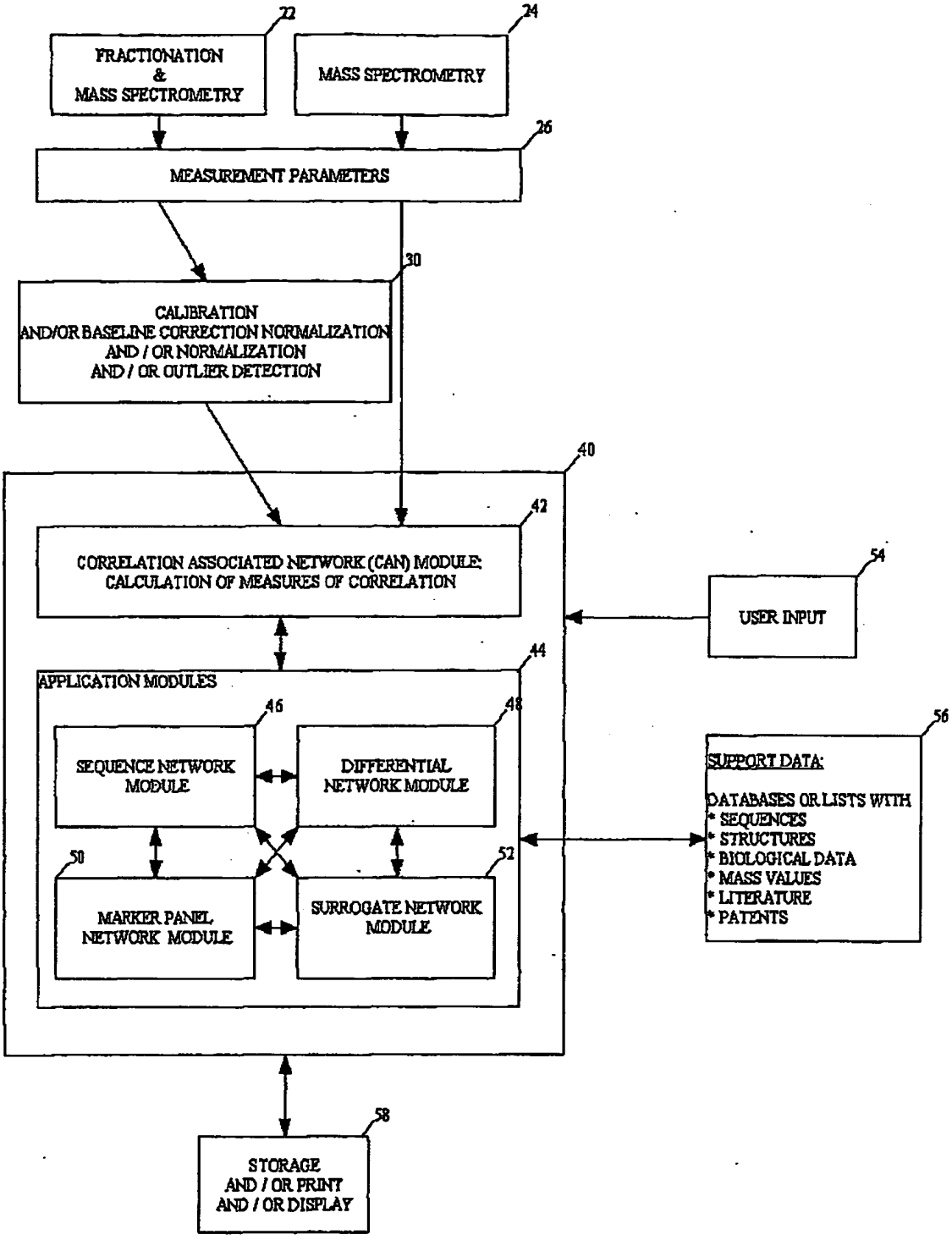

Fig. 1

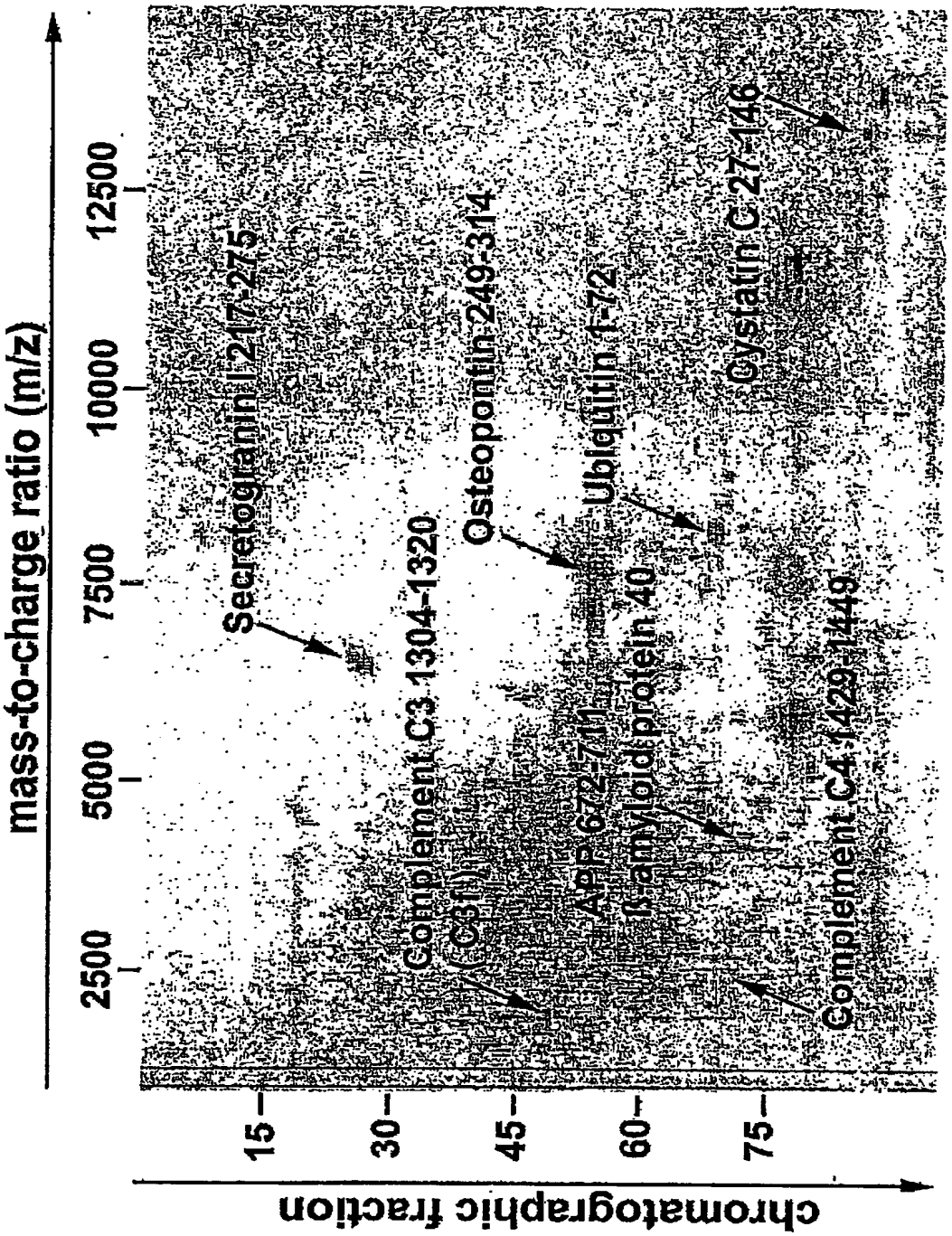
mass-to-charge ratio (m/z)
2500
5000
7500
10000
12500
chromatographic fraction
15
30
45
60
75
Secretogranin I 217-275
Complement C3 1304-1320
(C3f)
Osteopontin 249-314
Ubiquitin 1-72
APP 672-711
ß-amyloidprotein 40
Cystatin C 27-146
Complement C4 1429-1449

Fig. 2

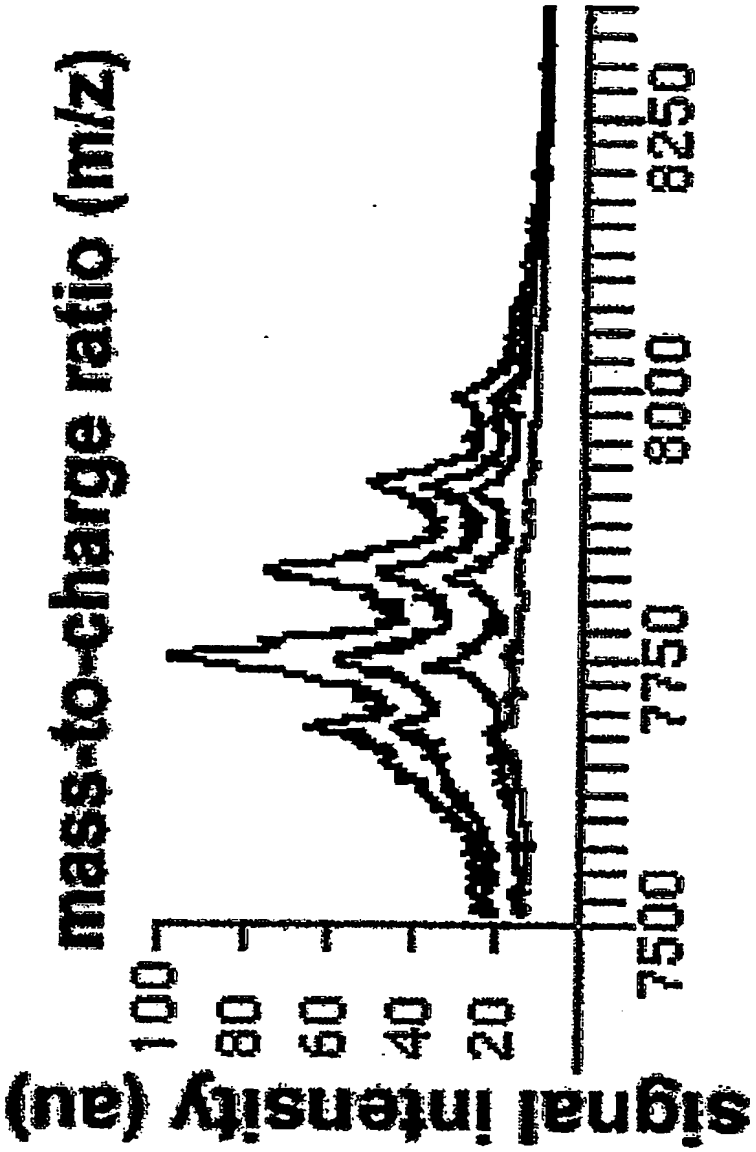
signal intensity (au)
100
80
60
40
20
mass-to-charge ratio (m/z)
7500
7750
8000
8250

Fig. 3

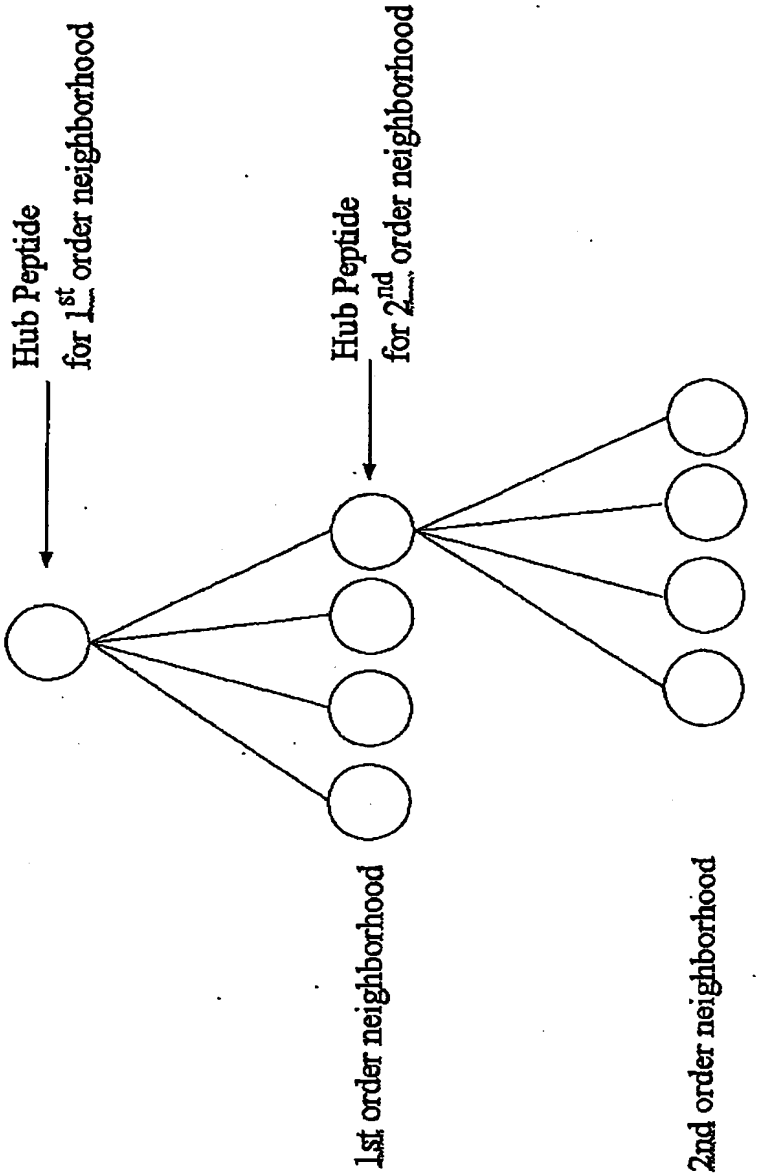
Hub Peptide
for 1st order neighborhood
Hub Peptide
for 2nd order neighborhood
1st order neighborhood
2nd order neighborhood

Fig. 4

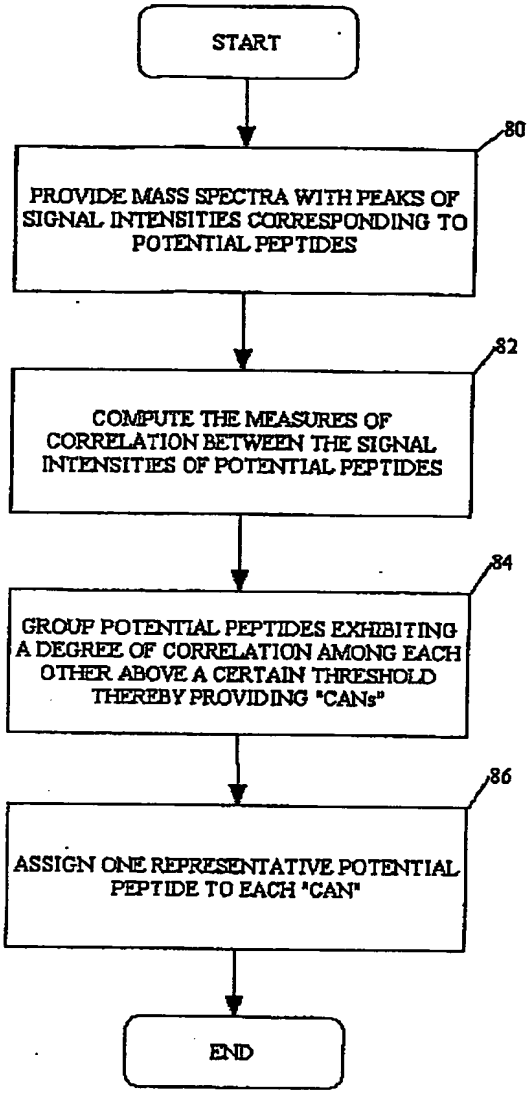
START
80
PROVIDE MASS SPECTRA WITH PEAKS OF SIGNAL INTENSITIES CORRESPONDING TO POTENTIAL PEPTIDES
82
COMPUTE THE MEASURES OF CORRELATION BETWEEN THE SIGNAL INTENSITIES OF POTENTIAL PEPTIDES
84
GROUP POTENTIAL PEPTIDES EXHIBITING A DEGREE OF CORRELATION AMONG EACH OTHER ABOVE A CERTAIN THRESHOLD THEREBY PROVIDING "CANs"
86
ASSIGN ONE REPRESENTATIVE POTENTIAL PEPTIDE TO EACH "CAN"
END

Fig. 5

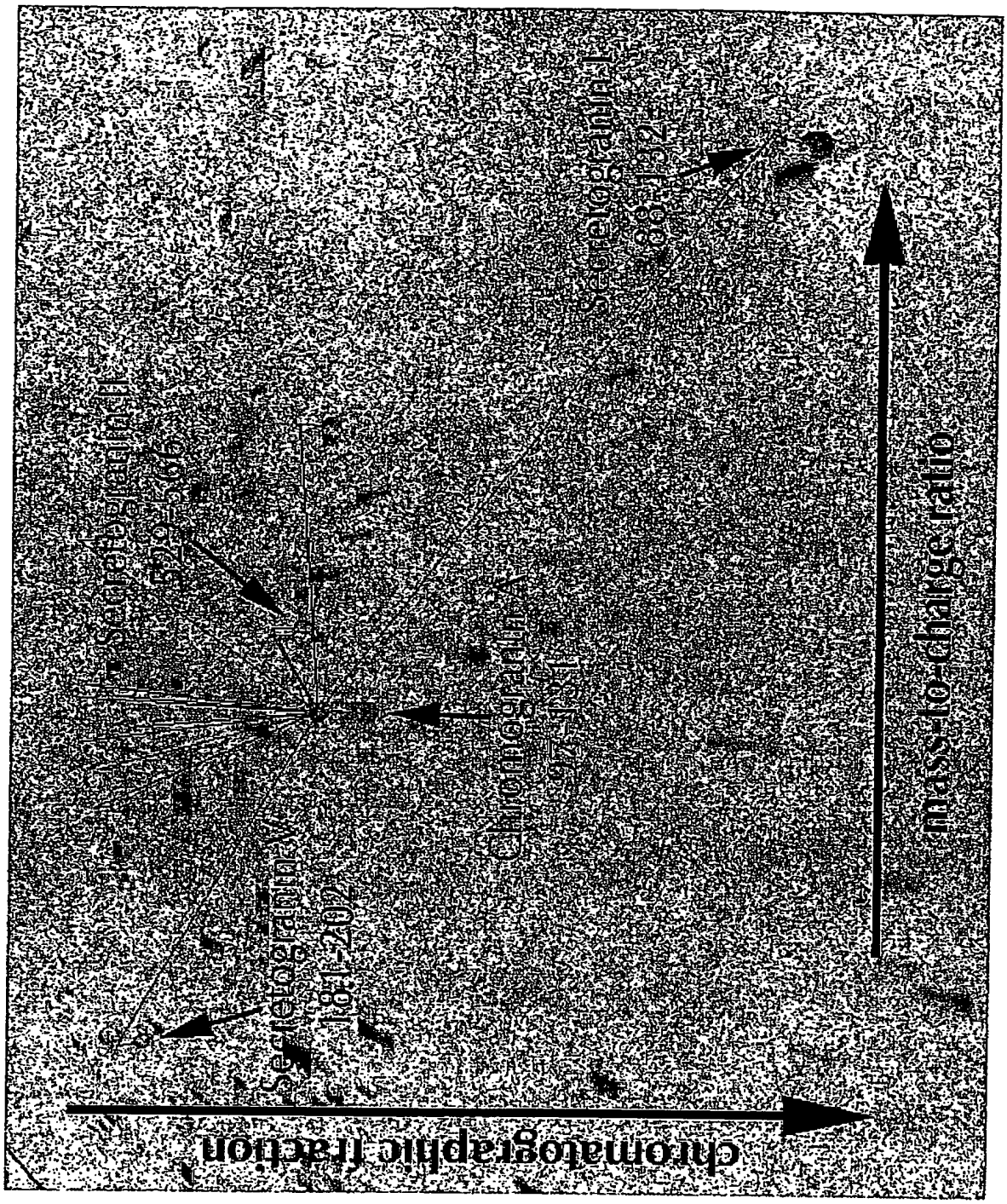

Fig. 6

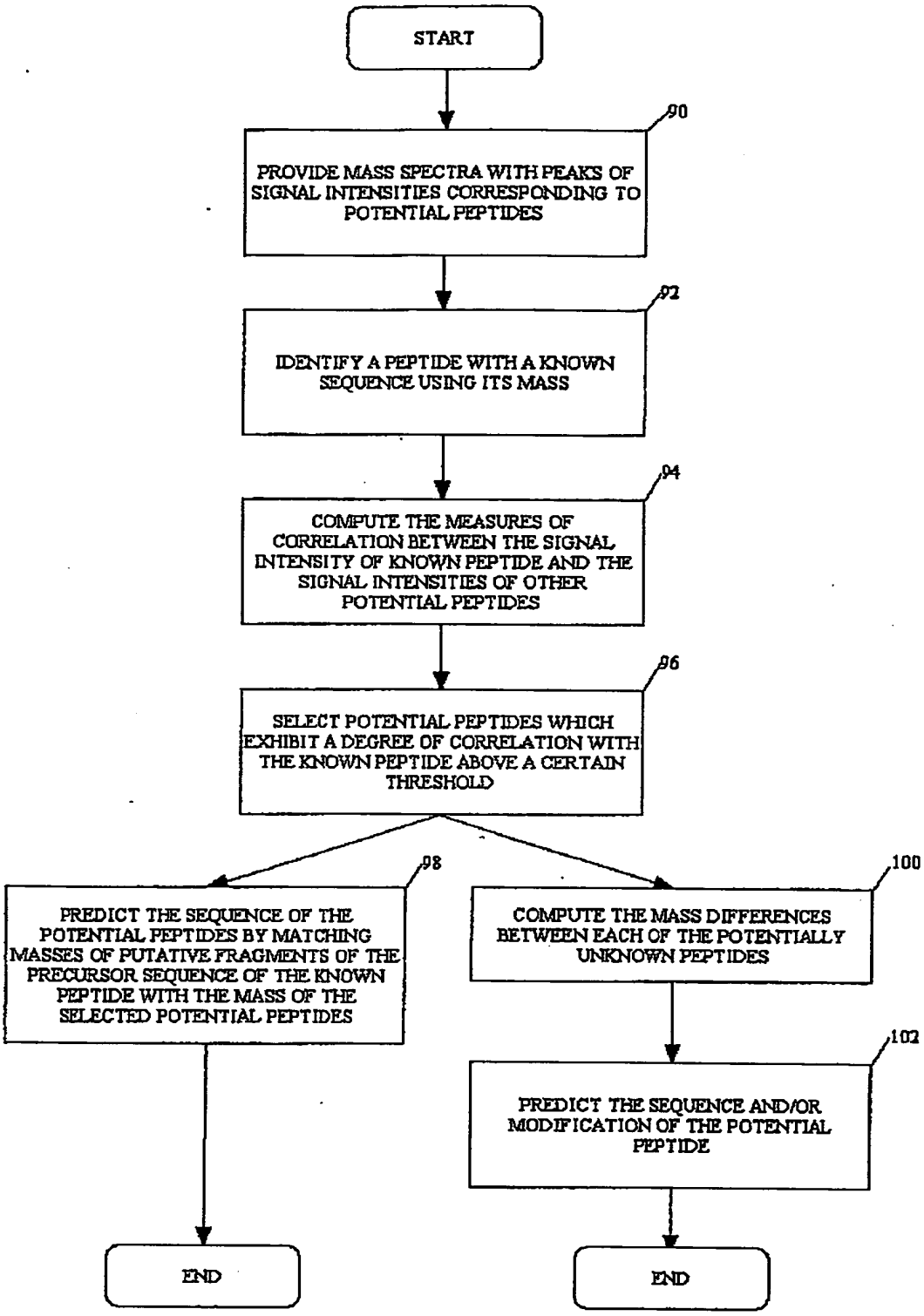
START
90
PROVIDE MASS SPECTRA WITH PEAKS OF SIGNAL INTENSITIES CORRESPONDING TO POTENTIAL PEPTIDES
92
IDENTIFY A PEPTIDE WITH A KNOWN SEQUENCE USING ITS MASS
94
COMPUTE THE MEASURES OF CORRELATION BETWEEN THE SIGNAL INTENSITY OF KNOWN PEPTIDE AND THE SIGNAL INTENSITIES OF OTHER POTENTIAL PEPTIDES
96
SELECT POTENTIAL PEPTIDES WHICH EXHIBIT A DEGREE OF CORRELATION WITH THE KNOWN PEPTIDE ABOVE A CERTAIN THRESHOLD
98
PREDICT THE SEQUENCE OF THE POTENTIAL PEPTIDES BY MATCHING MASSES OF PUTATIVE FRAGMENTS OF THE PRECURSOR SEQUENCE OF THE KNOWN PEPTIDE WITH THE MASS OF THE SELECTED POTENTIAL PEPTIDES
100
COMPUTE THE MASS DIFFERENCES BETWEEN EACH OF THE POTENTIALLY UNKNOWN PEPTIDES
102
PREDICT THE SEQUENCE AND/OR MODIFICATION OF THE POTENTIAL PEPTIDE
END
END

Fig. 7

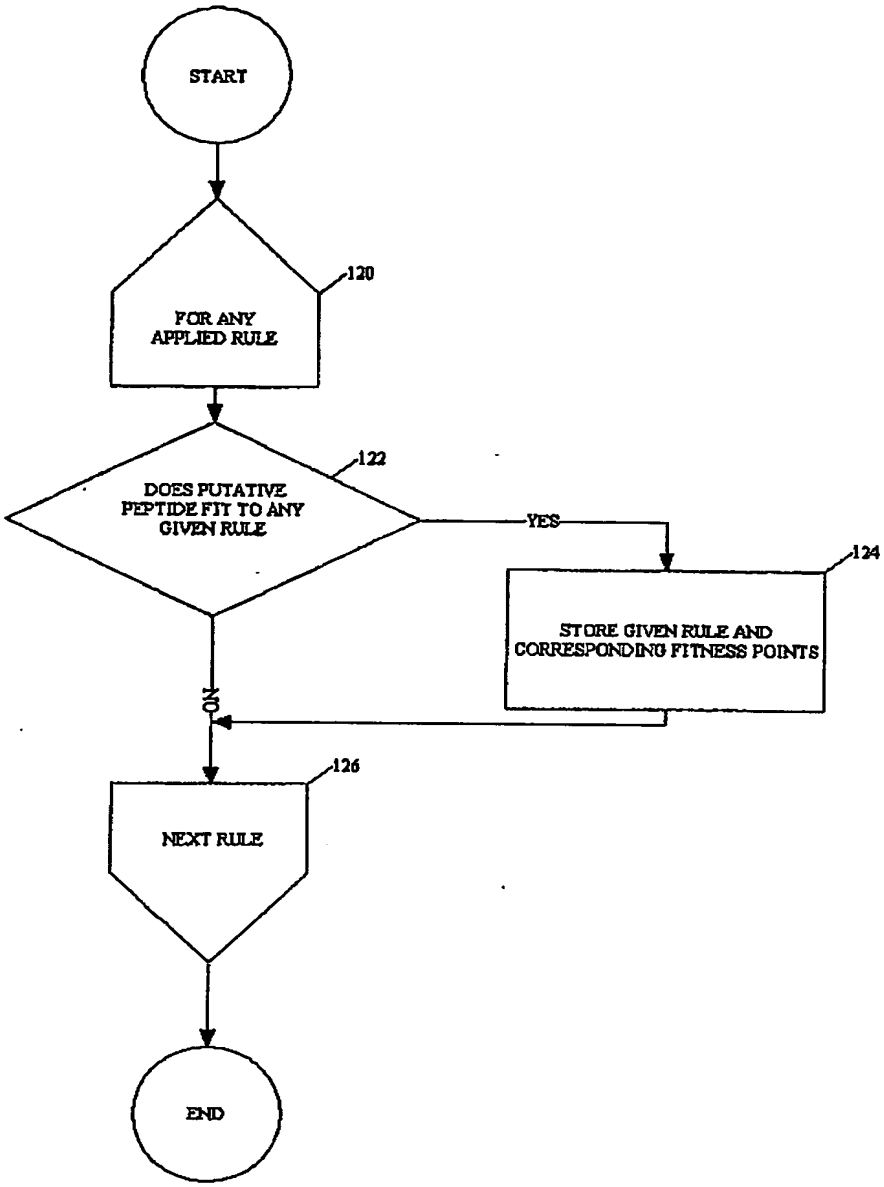
START
FOR ANY APPLIED RULE
120
DOES PUTATIVE PEPTIDE FIT TO ANY GIVEN RULE
122
YES
STORE GIVEN RULE AND CORRESPONDING FITNESS POINTS
124
NO
NEXT RULE
126
END

Fig. 8a

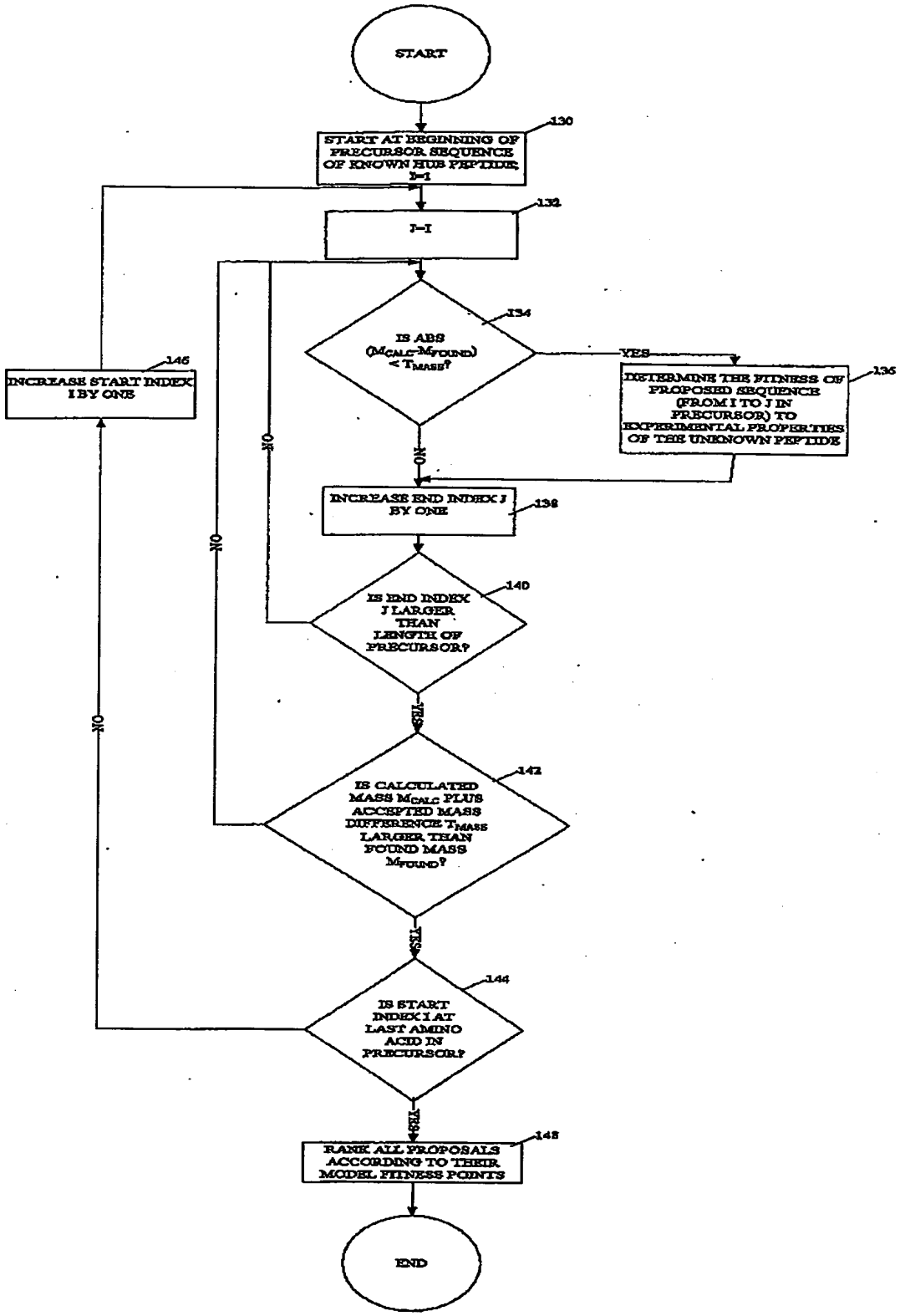
START
START AT BEGINNING OF PRECURSOR SEQUENCE OF KNOWN HUB PEPTIDE; I=1
130
J=I
132
IS ABS (M_{CALC}-M_{FOUND}) < T_{MASS}?
134
YES
DETERMINE THE FITNESS OF PROPOSED SEQUENCE (FROM I TO J IN PRECURSOR) TO EXPERIMENTAL PROPERTIES OF THE UNKNOWN PEPTIDE
136
NO
INCREASE START INDEX I BY ONE
146
INCREASE END INDEX J BY ONE
138
IS END INDEX J LARGER THAN LENGTH OF PRECURSOR?
140
NO
YES
IS CALCULATED MASS M_{CALC} PLUS ACCEPTED MASS DIFFERENCE T_{MASS} LARGER THAN FOUND MASS M_{FOUND}?
142
NO
YES
IS START INDEX I AT LAST AMINO ACID IN PRECURSOR?
144
NO
YES
RANK ALL PROPOSALS ACCORDING TO THEIR MODEL FITNESS POINTS
148
END

Fig. 8b

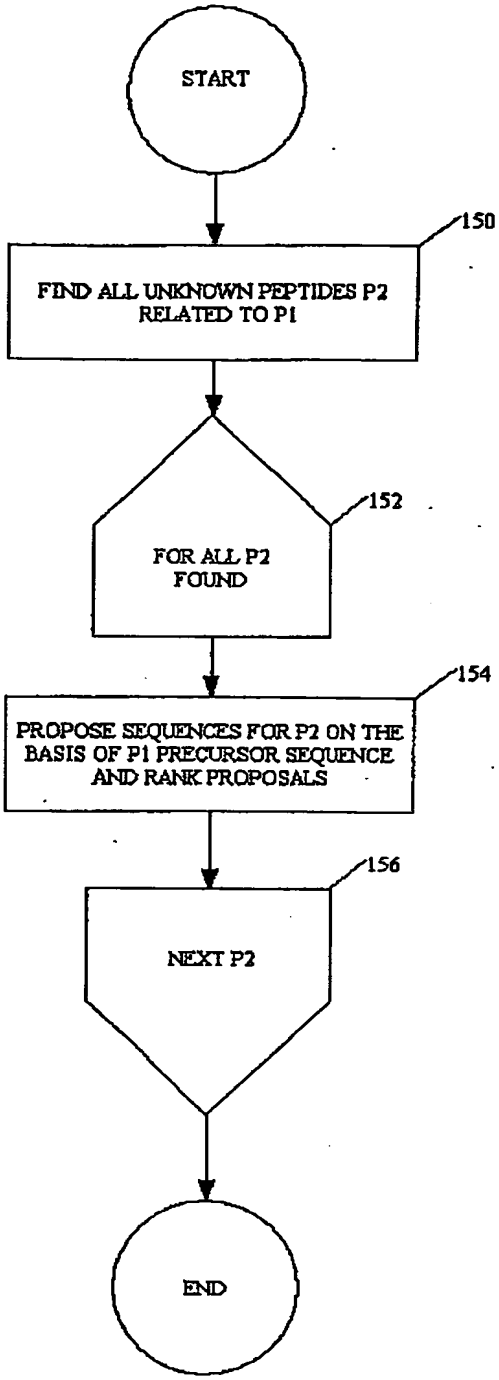
START
150
FIND ALL UNKNOWN PEPTIDES P2 RELATED TO P1
152
FOR ALL P2 FOUND
154
PROPOSE SEQUENCES FOR P2 ON THE BASIS OF P1 PRECURSOR SEQUENCE AND RANK PROPOSALS
156
NEXT P2
END

Fig. 8c

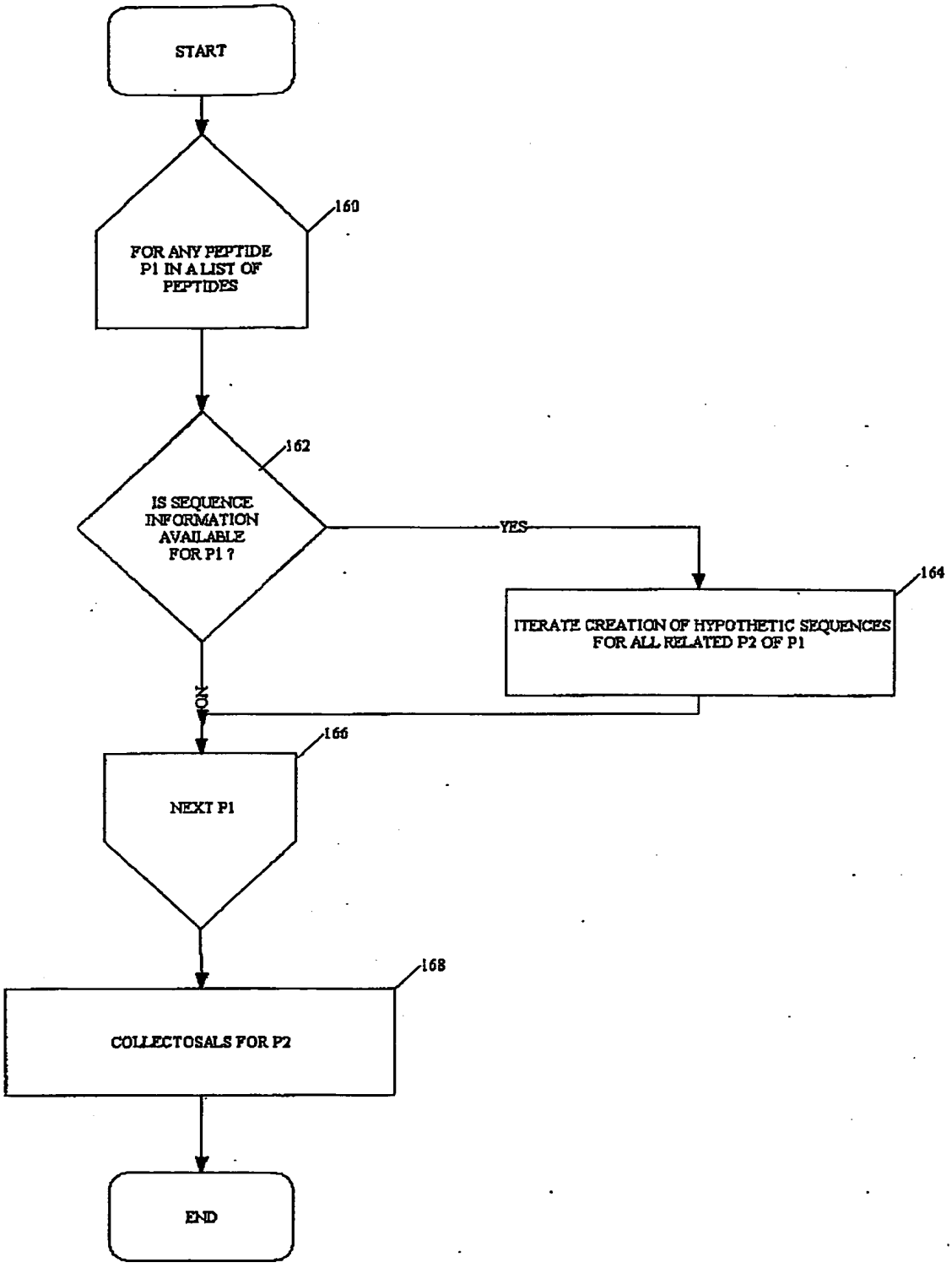
START
FOR ANY PEPTIDE P1 IN A LIST OF PEPTIDES
160
IS SEQUENCE INFORMATION AVAILABLE FOR P1 ?
162
YES
NO
ITERATE CREATION OF HYPOTHETIC SEQUENCES FOR ALL RELATED P2 OF P1
164
NEXT P1
166
COLLECTOSALS FOR P2
168
END

Fig. 8d

Modification	Monoisotopic Mass	Average Mass
4-Phosphopantetheine	339.0780	339.3294
5-Adenosylation	329.0525	329.2081
Acetylation	42.0106	42.0373
ADP-ribosylation (from NAD)	541.0611	541.3052
Biotinylation (amide bond to lysine)	226.0776	226.2994
Carboxylation of Asp and Glu	43.9898	44.0098
C-terminal amide formed from Gly	-0.9840	-0.9847
Cysteinylation	119.0041	119.1442
Deamidation of Asn and Gln	0.9840	0.9847
Deoxyhexoses (Fuc, Rha)	146.0579	146.1430
Disulphide bond formation	-2.0157	-2.0159
Farnesylation	204.1878	204.3556
Formylation	27.9949	28.0104
Ceramylation	272.2504	272.4741
Glutathionylation	305.0682	305.3117
Hexosamines (GalN, GlcN)	161.0688	161.1577
Hexoses (Fru, Gal, Glc, Man)	162.0528	162.1424
Homoserine formed from Met by CNBr treatment	-29.9928	-30.0935
Hydroxylation	15.9949	15.9994
Lipoic acid (amide bond to lysine)	188.0330	188.3147
Methylation	14.0157	14.0269
Myristoylation	210.1984	210.3598
N-acetylhexosamines (GalNAc, GlcNAc)	203.0794	203.1950
N-acetylneuraminic acid (Sialic acid, NeuAc, NANA, SA)	291.0954	291.2579
N-glycolylneuraminic acid (NeuGc)	307.0903	307.2573
Oxidation of Met	15.9949	15.9994
Palmitoylation	238.2297	238.4186
Pentoses (Ara, Rib, Xyl)	132.0423	132.1161
Phosphorylation	79.9663	79.9799
Proteolysis of a single peptide bond	18.0106	18.0153
Pyridoxal phosphate (Schiff Base formed to lysine)	231.0297	231.1449
Pyroglutamic acid formed from Gln	-17.0265	-17.0306
Stearoylation	266.2610	266.4674
Sulphation	79.9568	80.0642

Fig. 9

Motif	Enzyme/ Reaction	Mass difference(average mass)
W	photochemical	+16
W	photochemical	+32
W	photochemical	+4
[ST]-X-[RK]	Protein kinase C	+79.9799

Fig. 10

Symbols		Monoisotopic Mass	Average Mass
Gly	G	57.02146	57.05
Ala	A	71.03711	71.08
Ser	S	87.03202	87.08
Pro	P	97.05276	97.12
Val	V	99.06841	99.07
Thr	T	101.0476	101.1
Cys	C	103.0091	103.1
Leu	L	113.0840	113.2
Ile	I	113.0840	113.2
Asn	N	114.0429	114.1
Asp	D	115.0269	115.1
Gln	Q	128.0585	128.1
Lys	K	128.0949	128.2
Glu	E	129.0425	129.1
Met	M	131.0404	131.2
His	H	137.0589	137.1
Phe	F	147.0684	147.2
Arg	R	156.1011	156.2
Tyr	Y	163.0633	163.2
Trp	W	186.0793	186.2

Fig. 11

	Composition	Monoisotopic Mass	Average Mass
N-Terminal Groups			
Hydrogen	H	1.00782	1.0079
N-Formyl	HCO	29.00274	29.0183
N-Acetyl	CH_3CO	43.01839	43.0452
C-Terminal Groups			
Free acid	OH	17.00274	17.0073

Fig. 12

Additional Amino acid	+/- Fraction numbers
E, Glutamic acid	+ 0.47
F, Phenylalanine	+ 5.54
H, Histidine	- 3.82
I, Isoleucine	+ 2.86
K, Lysine	- 1.72
L, Leucine	+ 5.15
Q, Glutamine	- 0.85
S , Serine	- 0.45
V, Valine	+ 2.2
W, Tryptophane	+ 5.35
Y, Tyrosine	+ 2.92

Fig. 13

AminoAcid Before First Cleavage				
		% Cleavage	% Present	Δ
A	24	15.5%	8.4%	184%
R	32	20.6%	6.3%	329%
M	4	2.6%	1.5%	171%
W	3	1.9%	1.2%	168%
P	14	9.0%	6.2%	145%
N	7	4.5%	3.3%	135%
F	6	3.9%	3.2%	122%
K	10	6.5%	6.0%	107%
G	11	7.1%	6.6%	107%
L	12	7.7%	8.5%	91%
H	3	1.9%	2.7%	71%
V	5	3.2%	5.3%	61%
D	5	3.2%	5.5%	58%
Q	3	1.9%	4.6%	42%
C	1	0.6%	1.8%	36%
I	1	0.6%	2.1%	31%
T	2	1.3%	4.7%	27%
S	3	1.9%	7.8%	25%
E	4	2.6%	11.5%	22%
Y	0	0.0%	2.6%	0%
PrecursorStart	5	3.2%		

Fig. 14a

AminoAcid after First Cleavage				
		% Cleavage	% Present	Δ
S	26	16.8%	7.8%	214%
D	18	11.6%	5.5%	210%
V	13	8.4%	5.3%	158%
H	6	3.9%	2.7%	143%
G	14	9.0%	6.6%	136%
M	3	1.9%	1.5%	128%
I	4	2.6%	2.1%	124%
A	16	10.3%	8.4%	122%
K	9	5.8%	6.0%	97%
P	9	5.8%	6.2%	93%
Q	6	3.9%	4.6%	84%
L	11	7.1%	8.5%	83%
T	6	3.9%	4.7%	82%
N	3	1.9%	3.3%	58%
Y	2	1.3%	2.6%	50%
C	1	0.6%	1.8%	36%
E	5	3.2%	11.5%	28%
R	2	1.3%	6.3%	21%
F	1	0.6%	3.2%	20%
W	0	0.0%	1.2%	0%

Fig. 14b

AminoAcid Before Last Cleavage				
		% Cleavage	% Present	Δ
R	26	16.8%	6.3%	267%
E	29	18.7%	11.5%	162%
N	7	4.5%	3.3%	135%
D	11	7.1%	5.5%	128%
A	16	10.3%	8.4%	122%
Q	8	5.2%	4.6%	111%
R	10	6.5%	6.3%	103%
F	5	3.2%	3.2%	102%
L	13	8.4%	8.5%	98%
G	10	6.5%	6.6%	97%
K	9	5.8%	6.0%	97%
M	2	1.3%	1.5%	85%
T	6	3.9%	4.7%	82%
S	9	5.8%	7.8%	74%
C	2	1.3%	1.8%	72%
V	5	3.2%	5.3%	61%
Y	2	1.3%	2.6%	50%
H	2	1.3%	2.7%	48%
P	2	1.3%	6.2%	21%
I	0	0.0%	2.1%	0%

Fig. 14c

AminoAcid After Last Cleavage				
		% Cleavage	% Present	Δ
Precursor end	**30**	**19.4%**		
R	**26**	**16.8%**	**6.3%**	**267%**
K	**19**	**12.3%**	**6.0%**	**204%**
W	3	1.9%	1.2%	168%
F	8	5.2%	3.2%	162%
G	12	7.7%	6.6%	117%
V	9	5.8%	5.3%	110%
T	7	4.5%	4.7%	96%
I	2	1.3%	2.1%	62%
A	7	4.5%	8.4%	54%
P	5	3.2%	6.2%	52%
Y	2	1.3%	2.6%	50%
M	1	0.6%	1.5%	43%
Q	3	1.9%	4.6%	42%
S	5	3.2%	7.8%	41%
N	2	1.3%	3.3%	39%
L	5	3.2%	8.5%	38%
D	3	1.9%	5.5%	35%
E	6	3.9%	11.5%	34%
C	0	0.0%	1.8%	0%
H	0	0.0%	2.7%	0%

Fig. 14d

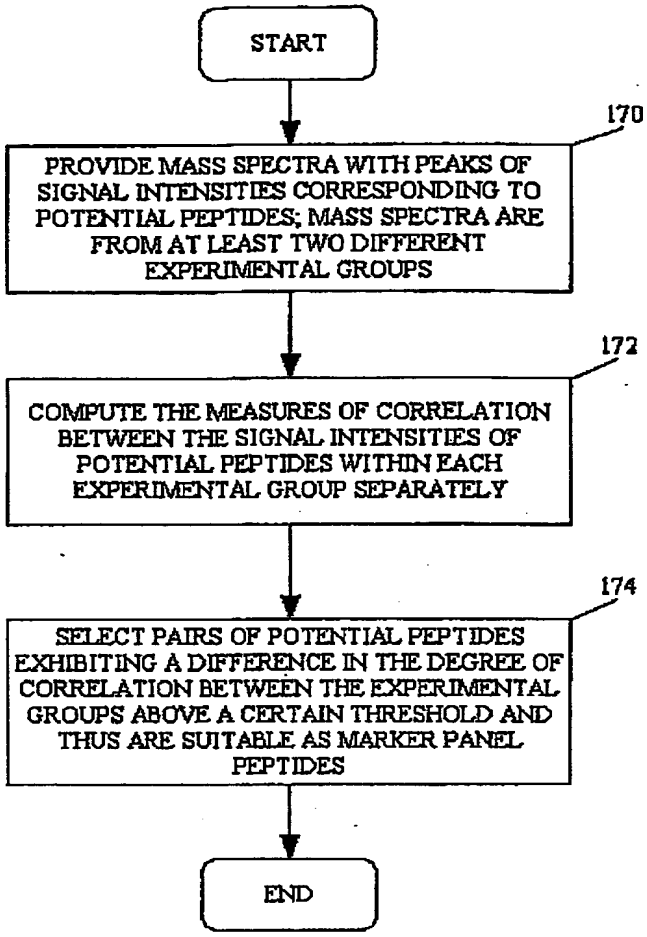
START
170
PROVIDE MASS SPECTRA WITH PEAKS OF SIGNAL INTENSITIES CORRESPONDING TO POTENTIAL PEPTIDES; MASS SPECTRA ARE FROM AT LEAST TWO DIFFERENT EXPERIMENTAL GROUPS
172
COMPUTE THE MEASURES OF CORRELATION BETWEEN THE SIGNAL INTENSITIES OF POTENTIAL PEPTIDES WITHIN EACH EXPERIMENTAL GROUP SEPARATELY
174
SELECT PAIRS OF POTENTIAL PEPTIDES EXHIBITING A DIFFERENCE IN THE DEGREE OF CORRELATION BETWEEN THE EXPERIMENTAL GROUPS ABOVE A CERTAIN THRESHOLD AND THUS ARE SUITABLE AS MARKER PANEL PEPTIDES
END

Fig. 15

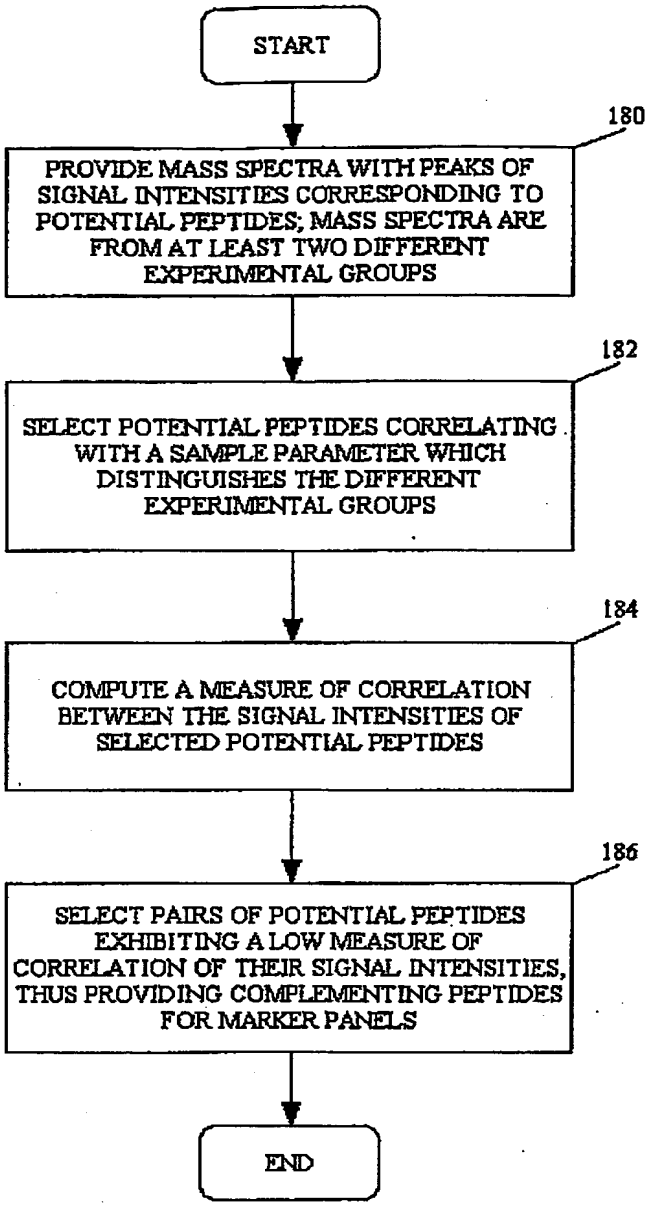
START
180
PROVIDE MASS SPECTRA WITH PEAKS OF SIGNAL INTENSITIES CORRESPONDING TO POTENTIAL PEPTIDES; MASS SPECTRA ARE FROM AT LEAST TWO DIFFERENT EXPERIMENTAL GROUPS
182
SELECT POTENTIAL PEPTIDES CORRELATING WITH A SAMPLE PARAMETER WHICH DISTINGUISHES THE DIFFERENT EXPERIMENTAL GROUPS
184
COMPUTE A MEASURE OF CORRELATION BETWEEN THE SIGNAL INTENSITIES OF SELECTED POTENTIAL PEPTIDES
186
SELECT PAIRS OF POTENTIAL PEPTIDES EXHIBITING A LOW MEASURE OF CORRELATION OF THEIR SIGNAL INTENSITIES, THUS PROVIDING COMPLEMENTING PEPTIDES FOR MARKER PANELS
END

Fig. 16

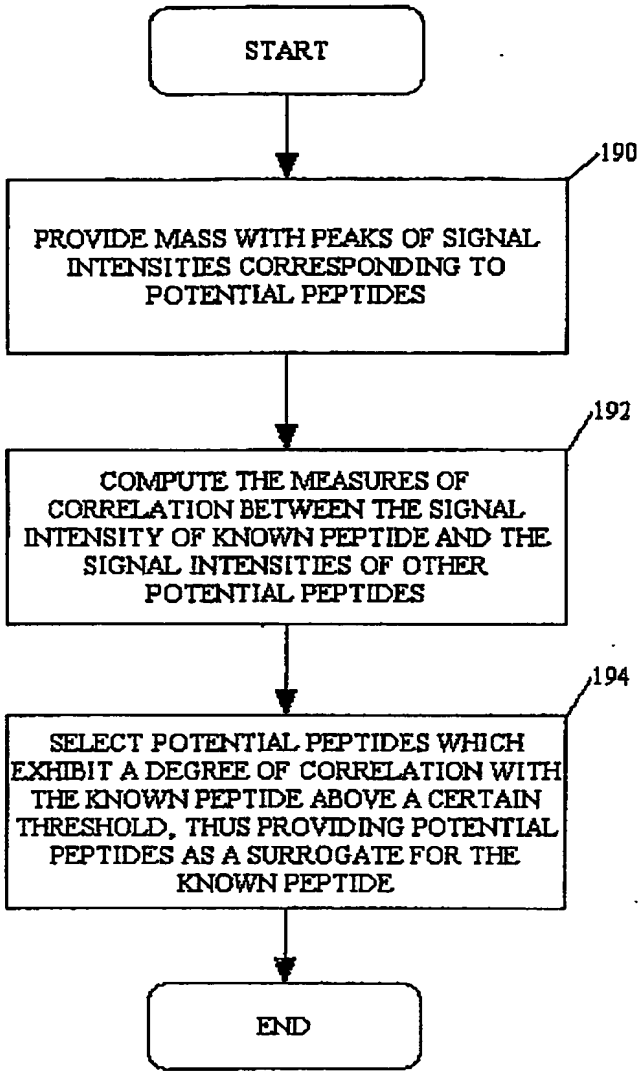
START
190
PROVIDE MASS WITH PEAKS OF SIGNAL INTENSITIES CORRESPONDING TO POTENTIAL PEPTIDES
192
COMPUTE THE MEASURES OF CORRELATION BETWEEN THE SIGNAL INTENSITY OF KNOWN PEPTIDE AND THE SIGNAL INTENSITIES OF OTHER POTENTIAL PEPTIDES
194
SELECT POTENTIAL PEPTIDES WHICH EXHIBIT A DEGREE OF CORRELATION WITH THE KNOWN PEPTIDE ABOVE A CERTAIN THRESHOLD, THUS PROVIDING POTENTIAL PEPTIDES AS A SURROGATE FOR THE KNOWN PEPTIDE
END

Fig. 17

Case No.	Fraction 54 m/z 2743.0	Fraction 54 m/z 1371.5	Fraction 56 m/z 2927.2	....	Fraction 20 m/z 11143	....
	k=20	k=19	k=16		k=19	
1	21648	712	2620		1452	
2	1830	1320	1199		2554	
3	21353	2022	3159		2139	
4	24223	2454	2431		2169	
5	3725	2719	982		1038	
6	13548	2956	1689		1300	
7	16606	3260	1402		2213	
8	20902	3435	624		887	
9	10321	3444	427		2145	
10	31047	3498	2238		1516	
11	31142	3592	4405		1255	
12	37241	3745	2785		1739	
13	22656	3822	2264		2576	
14	24366	3852	1752		1139	
15	16638	3935	1147		2182	
16	37171	4092	2393		1069	
17	33188	4115	1578		1681	
18	27596	4127	3228		1463	
19	39668	4348	3604		318	
20	12983	4362	1048		3039	
21	14420	4488	899		2676	
22	23261	4634	1965		3071	
23	30507	4710	2715		1247	
24	41494	4928	5343		1238	
25	36664	5107	3914		3156	
26	42465	5135	2729		1768	
27	42551	5135	3010		2500	
28	35473	5201	2242		1978	
29	48611	5906	2381		1075	
30	28413	5914	1855		3189	
31	35258	5954	3368		2140	
32	44774	6056	4167		670	
33	46137	6465	7640		1719	
34	40892	6531	1630		1241	
35	48202	7076	11222		3826	
36	43760	7183	4771		1565	
37	50211	7316	5443		2060	

Fig. 18a

Case No.	Fraction 54 m/z 2743.0	Fraction 54 m/z 1371.5	Fraction 56 m/z 2927.2	...	Fraction 20 m/z 1114.3	...
38	49824	7410	3004		1113	
39	50785	7752	6412		1616	
40	46200	7821	3689		3725	
41	52471	7949	5395		1837	
42	49299	8280	4623		1207	
43	45032	8483	4881		1566	
44	51224	8562	6481		2194	
45	51901	8638	10081		2047	
46	51084	8776	14193		1478	
47	50928	8852	6635		287	
48	50707	10097	8877		1458	
49	52304	10259	6244		1860	
50	48355	10661	5195		3695	
51	51363	10685	11403		1261	
52	54423	10846	11299		2067	
53	55167	11041	12868		1545	
54	55091	11539	5597		2381	
55	56825	11912	7718		2409	
56	53173	12022	8865		1969	
57	51649	12057	7855		1295	
58	51328	12095	9035		2043	
59	53464	12641	6408		856	
60	54542	12891	10363		1858	
61	56950	13172	7586		1802	
62	43273	14559	20080		596	
63	57335	14922	12288		2916	
64	55118	14997	10078		1761	
65	57147	16164	7726		2626	
66	55584	16216	17106		2623	
67	59414	16550	15122		539	
68	57093	16689	19689		2078	
69	57841	18254	16079		1659	
70	54084	18734	19524		395	
71	56325	22730	10828		2326	
72	58386	24159	16681		1631	
73	54843	26671	44356		3183	
74	53935	27937	30189		1403	

Fig. 18b

	Fraction 54 m/z 2743.0 and ...		
Measure of Association	Fraction 54 m/z 1371.5	Fraction 56 m/ 2927.2	Fraction 20 m/z 1114.3
Spearman's rank order correlation	0.9298	0.8761	-0.0044
Pearson's product moment correlation	0.7318	0.5855	-0.0781
Kendall's rank correlation tau	0.7704	0.6919	0.0107
MST diameter	50	40	29

Fig. 19

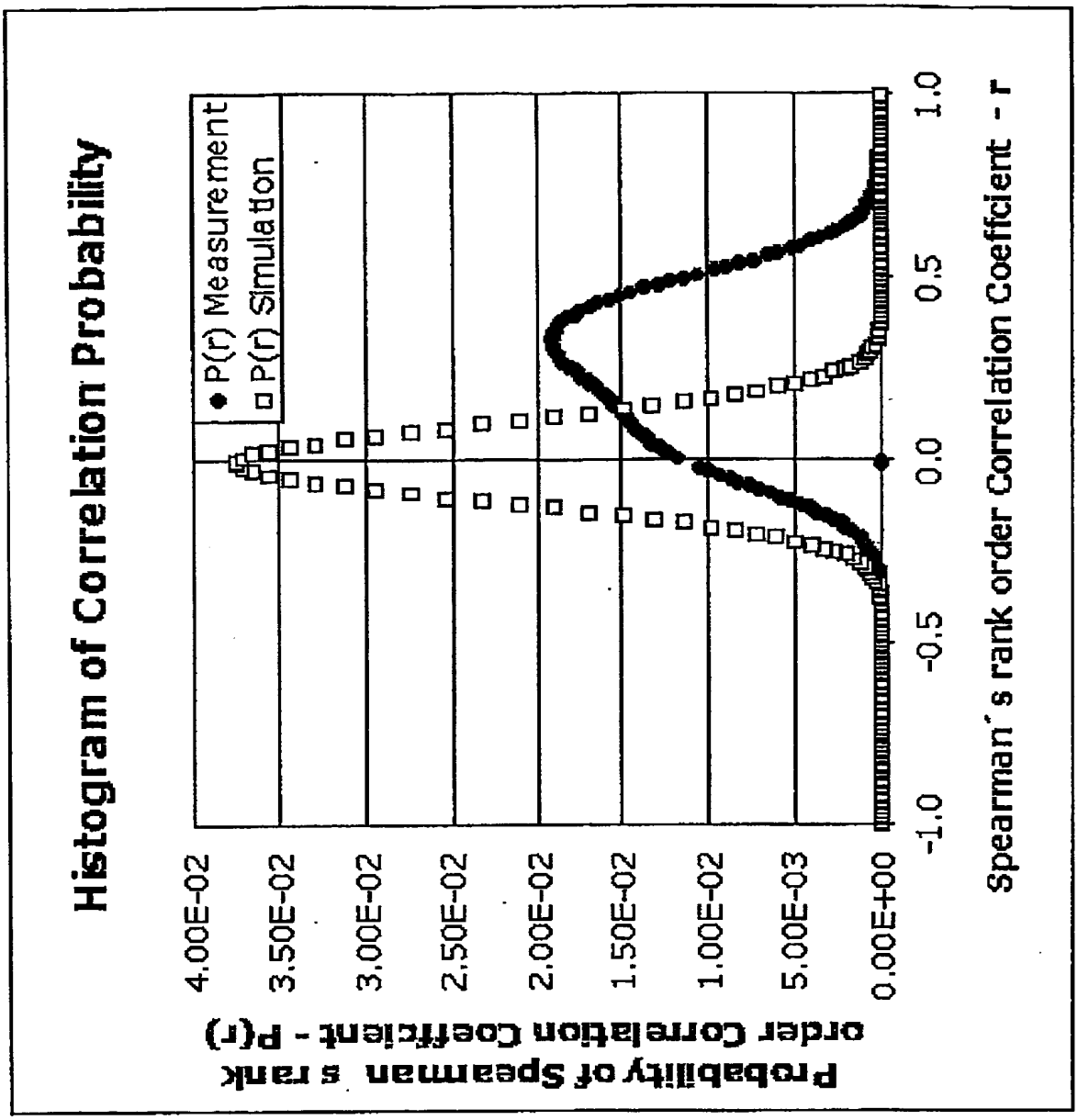
Histogram of Correlation Probability
P(r) Measurement
P(r) Simulation
Probability of Spearman s rank order Correlation Coefficient - P(r)
4.00E-02
3.50E-02
3.00E-02
2.50E-02
2.00E-02
1.50E-02
1.00E-02
5.00E-03
0.00E+00
-1.0
-0.5
0.0
0.5
1.0
Spearman´s rank order Correlation Coefficient - r

Fig. 20

Hub - Peptide	Correlation	Related peptide	Relative Mono-isotopic mass [Da]	Amino Acid Sequence
Chromogranin A 97 - 131			3905.764	HSGF EDELSEVLEN QSSQAELKEA VEEPSSKDVM E
	r=0.67	Secretogranin I 88-132	4605.025	DPADASEAHESSSR GEAGAP GEEDIQGPTKADTEKWAEGG GHSRE
	r=0.71	Secretogranin II 529-566	4152.921	G QGSSEDDLQEE EQIEQAIKEH LNQGSSQETD KLAPVS
	r=0.72	Secretogranin V 181-202	2448.334	SVNPYLQGQRLDNVVAKKSV PH

Fig. 21

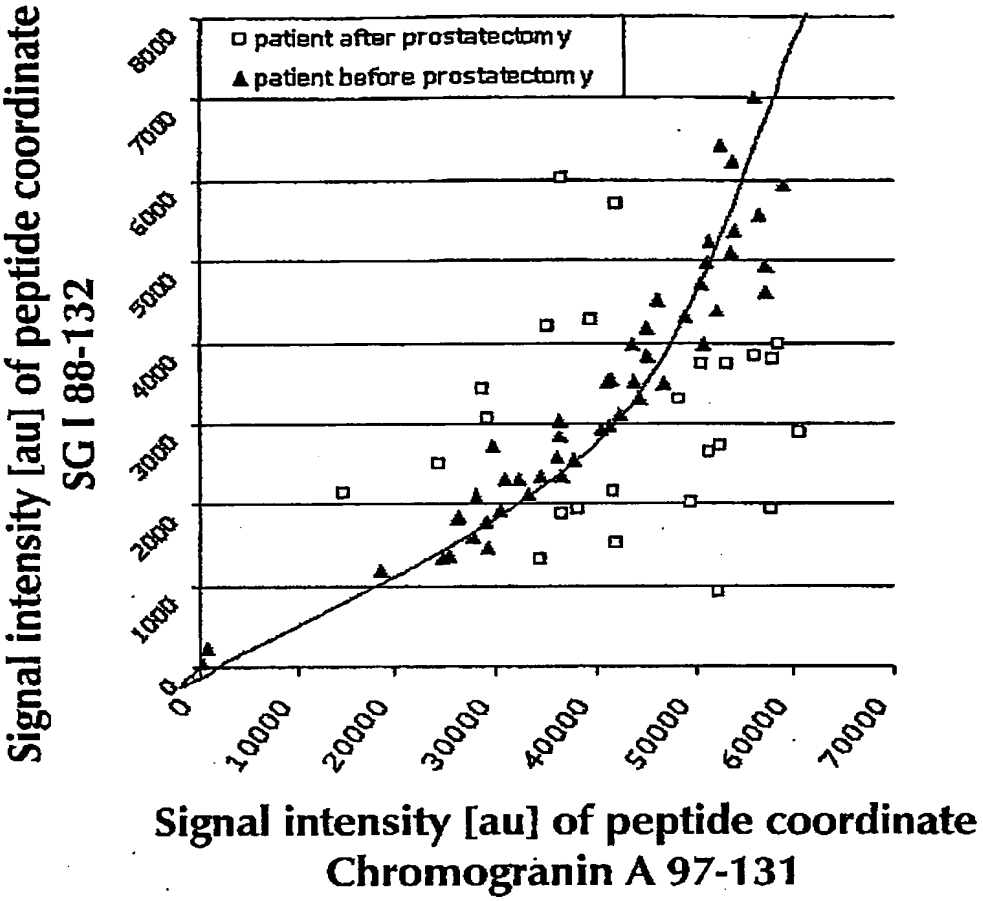
Signal intensity [au] of peptide coordinate SG I 88-132
patient after prostatectomy
patient before prostatectomy
0
1000
2000
3000
4000
5000
6000
7000
8000
0
10000
20000
30000
40000
50000
60000
70000
Signal intensity [au] of peptide coordinate Chromogranin A 97-131

Fig. 22

	Fraction 54 m/z 2743.8	Fraction 54 m/z 1371.5	Fraction 56 m/z 2927.2	....	Fraction 20 m/z 1114.3	....
	k=0	k=1	k=2		k=19	
case 1	21648	3747	7051		1452	
case 2	1830	4355	5630		2554	
case 3	21953	5057	7590		2139	
case 4	24223	5489	6862		2169	
case 5	3725	5754	5413		1038	
case 6	13548	5991	6120		1300	
case 7	16686	6295	5833		2213	
case 8	28982	6470	5055		887	
case 9	10321	6479	4858		2145	
case 10	31047	6533	6669		1516	
case 11	31142	6627	8836		1255	
case 12	37241	6780	7216		1739	
case 13	22656	6857	6695		2576	
case 14	24366	6887	6183		1139	
case 15	16628	6970	5578		2182	
case 16	37171	7127	6824		1069	
case 17	33188	7150	6009		1681	
case 18	27596	7162	7659		1463	
case 19	39668	7383	8035		318	
case 20	12983	7397	5479		3039	
case 21	14420	7523	5330		2676	
case 22	23261	7669	6396		3071	
case 23	30587	7745	7146		1247	
case 24	41494	7963	9774		1238	
case 25	36664	8142	8345		3156	
case 26	42465	8170	7160		1768	
case 27	42551	8170	7441		2500	
case 28	35473	8236	6673		1978	
case 29	48611	8941	6812		1075	
case 30	28413	8949	6286		3189	
case 31	39258	8989	7799		2140	
case 32	44774	9091	8598		670	
case 33	46137	9500	12071		1719	
case 34	40892	9566	6061		1241	
case 35	48202	10111	15653		3826	
case 36	43768	10218	9202		1565	

Fig. 23a

	Fraction 54 m/z 2743.0	Fraction 54 m/z 1371.5	Fraction 56 m/z 2927.2	....	Fraction 20 m/z 1114.3	....
case 37	50211	10351	9874		1113	
case 38	49804	10445	7435		1616	
case 39	58785	10787	10843		3725	
case 40	46208	10856	8120		1837	
case 41	52471	10984	9826		1207	
case 42	49299	11315	9054		1566	
case 43	49832	11518	9312		2194	
case 44	51224	11597	10912		2047	
case 45	51901	11673	14512		1478	
case 46	51084	11811	18624		287	
case 47	50928	11887	11066		1458	
case 48	50787	13132	13308		1860	
case 49	52304	13294	10675		3695	
case 50	48355	13696	9626		1261	
case 51	51363	13720	15834		2067	
case 52	54423	13881	15730		1545	
case 53	55167	14076	17299		2381	
case 54	55091	14574	10028		2409	
case 55	56825	14947	12149		1969	
case 56	53173	15057	13296		1295	
case 57	51649	15092	12286		2043	
case 58	51328	15130	13466		856	
case 59	53464	15676	10839		1858	
case 60	54542	15926	14794		1802	
case 61	56950	16207	12017		596	
case 62	42273	17594	24511		2916	
case 63	57335	17957	16719		1761	
case 64	55118	18032	14509		2626	
case 65	57147	19199	12157		2623	
case 66	55584	19251	21537		539	
case 67	59414	19585	19553		2078	
case 68	57093	19724	24120		1659	
case 69	57841	21289	20510		395	
case 70	54084	21769	23955		2326	
case 71	56325	25765	15259		1631	
case 72	58286	27194	21112		3183	
case 73	54842	29706	48787		1403	
case 74	53935	30972	34620		1113	

Fig. 23b

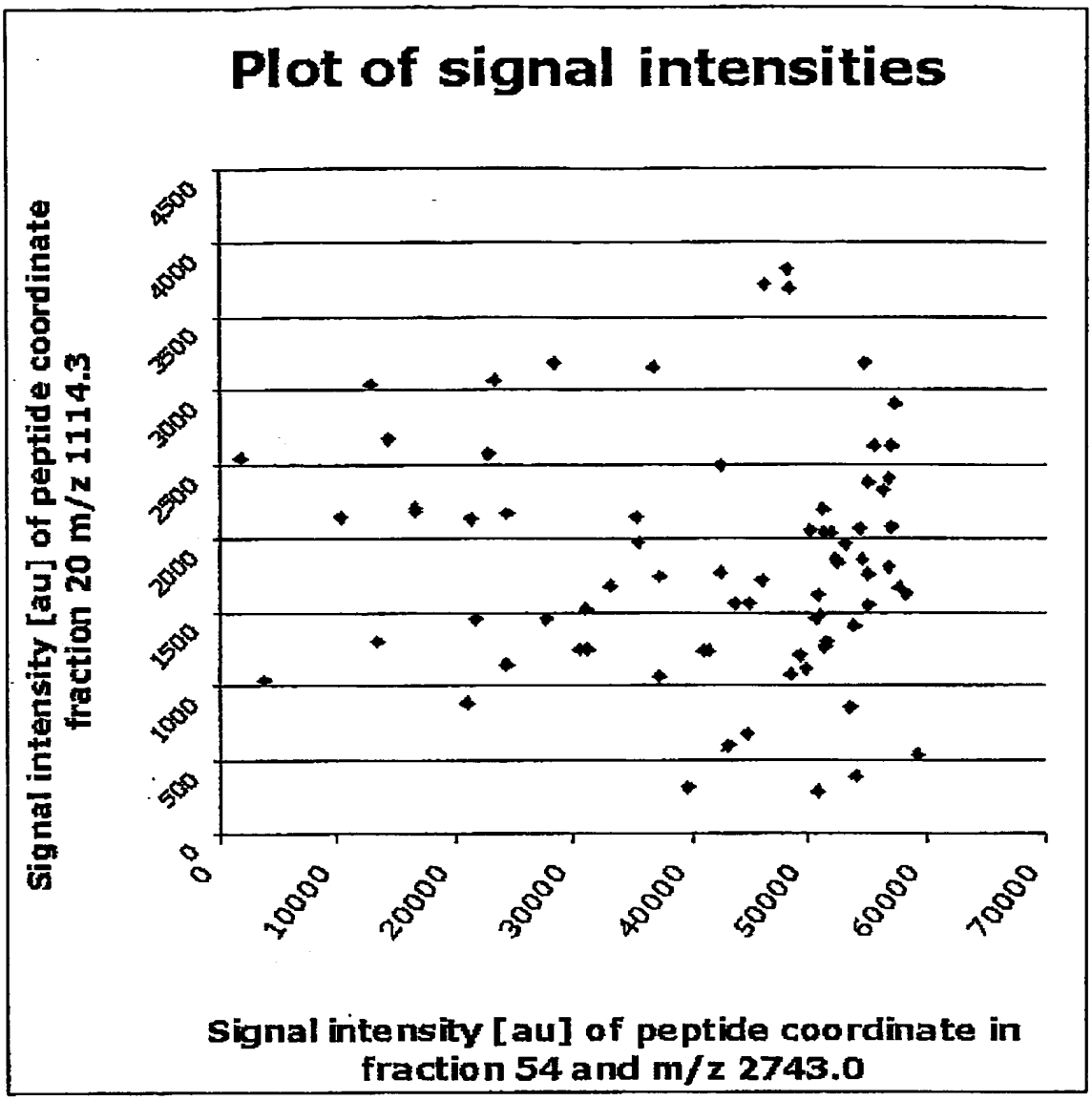
Plot of signal intensities
Signal intensity [au] of peptide coordinate fraction 20 m/z 1114.3
0
500
1000
1500
2000
2500
3000
3500
4000
4500
0
10000
20000
30000
40000
50000
60000
70000
Signal intensity [au] of peptide coordinate in fraction 54 and m/z 2743.0

Fig. 24a

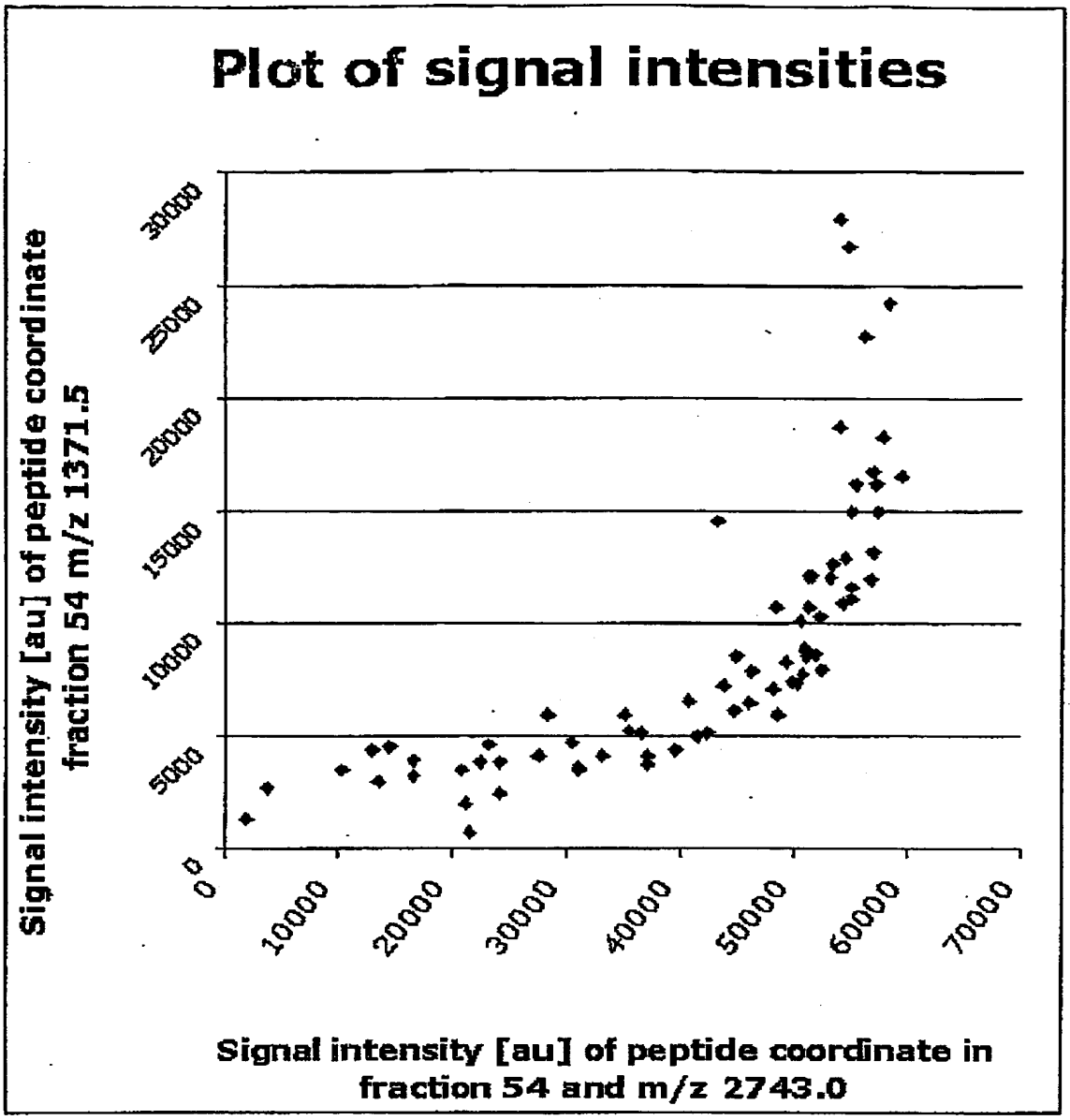
Plot of signal intensities
Signal intensity [au] of peptide coordinate fraction 54 m/z 1371.5
0
5000
10000
15000
20000
25000
30000
0
10000
20000
30000
40000
50000
60000
70000
Signal intensity [au] of peptide coordinate in fraction 54 and m/z 2743.0

Fig. 24b

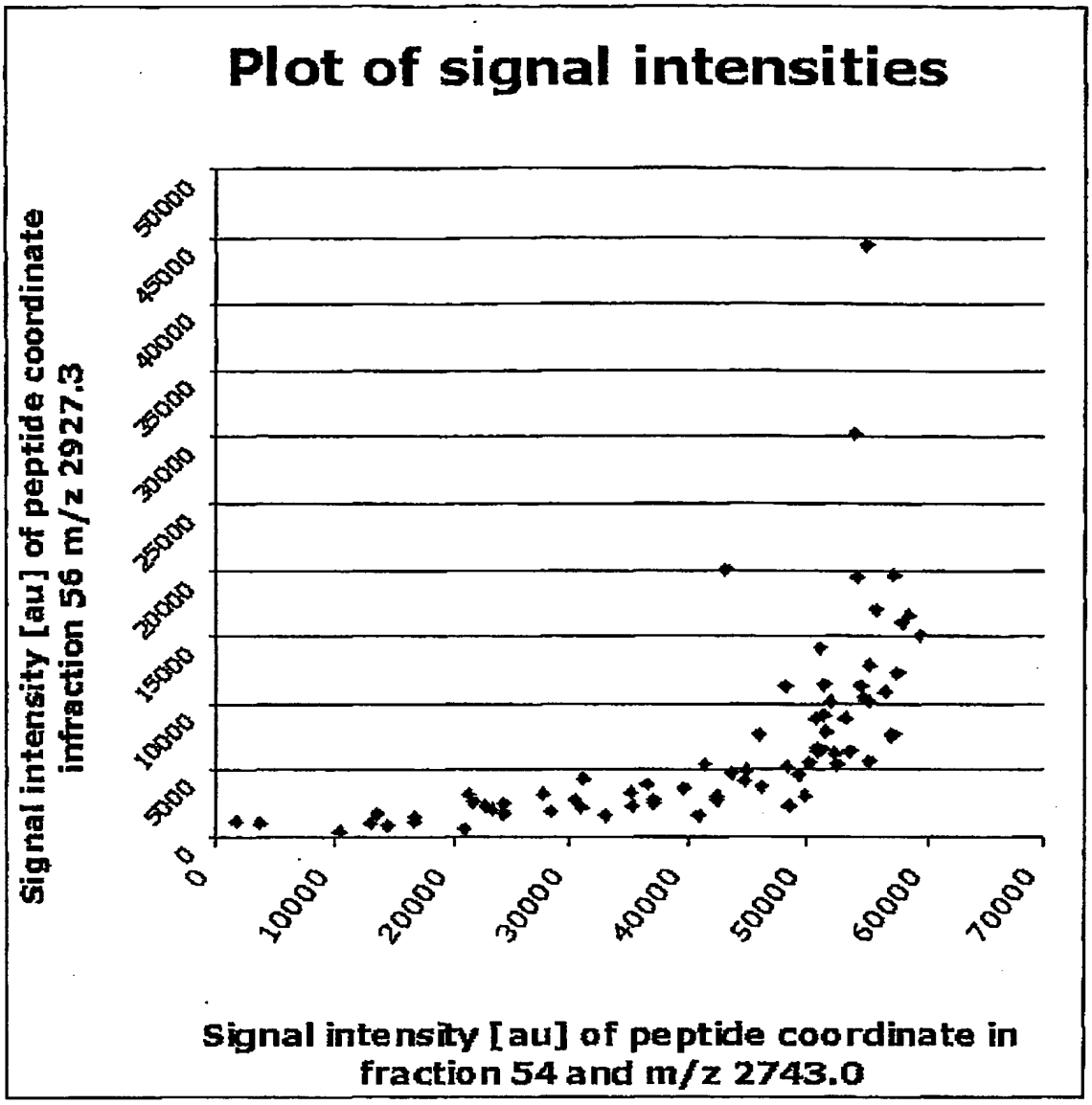
Plot of signal intensities
Signal intensity [au] of peptide coordinate infraction 56 m/z 2927.3
0
5000
10000
15000
20000
25000
30000
35000
40000
45000
50000
0
10000
20000
30000
40000
50000
60000
70000
Signal intensity [au] of peptide coordinate in fraction 54 and m/z 2743.0

Fig. 24c

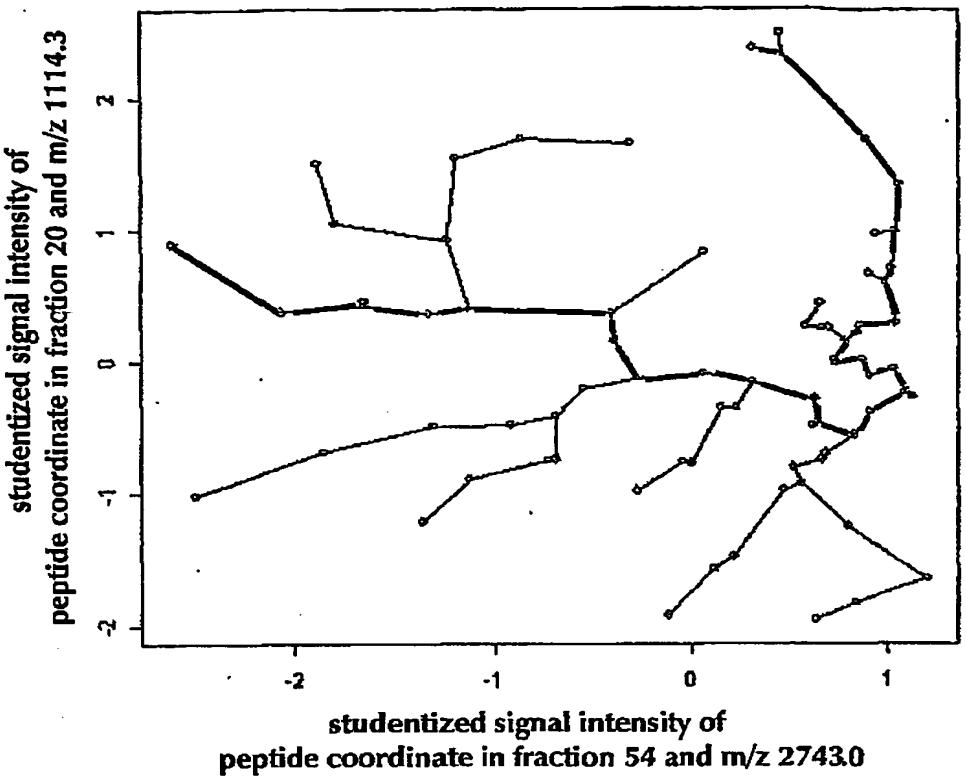
studentized signal intensity of
peptide coordinate in fraction 20 and m/z 1114.3
2
1
0
-1
-2
-2
-1
0
1
studentized signal intensity of
peptide coordinate in fraction 54 and m/z 2743.0

Fig. 25a

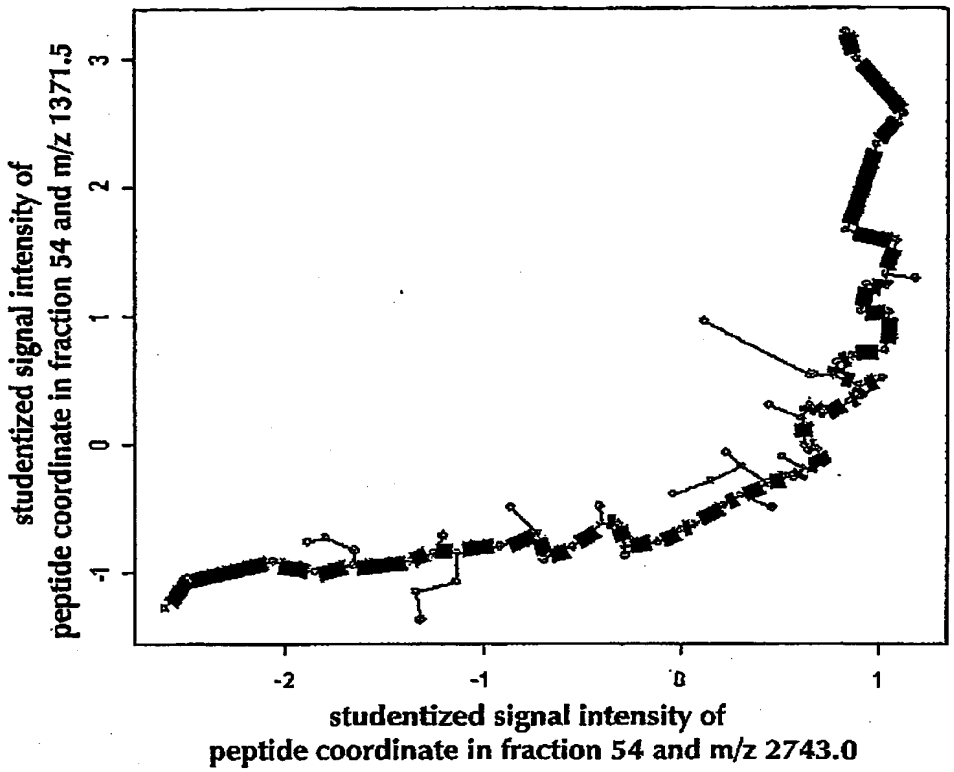
studentized signal intensity of
peptide coordinate in fraction 54 and m/z 1371.5
-1
0
1
2
3
-2
-1
0
1
studentized signal intensity of
peptide coordinate in fraction 54 and m/z 2743.0

Fig. 25b

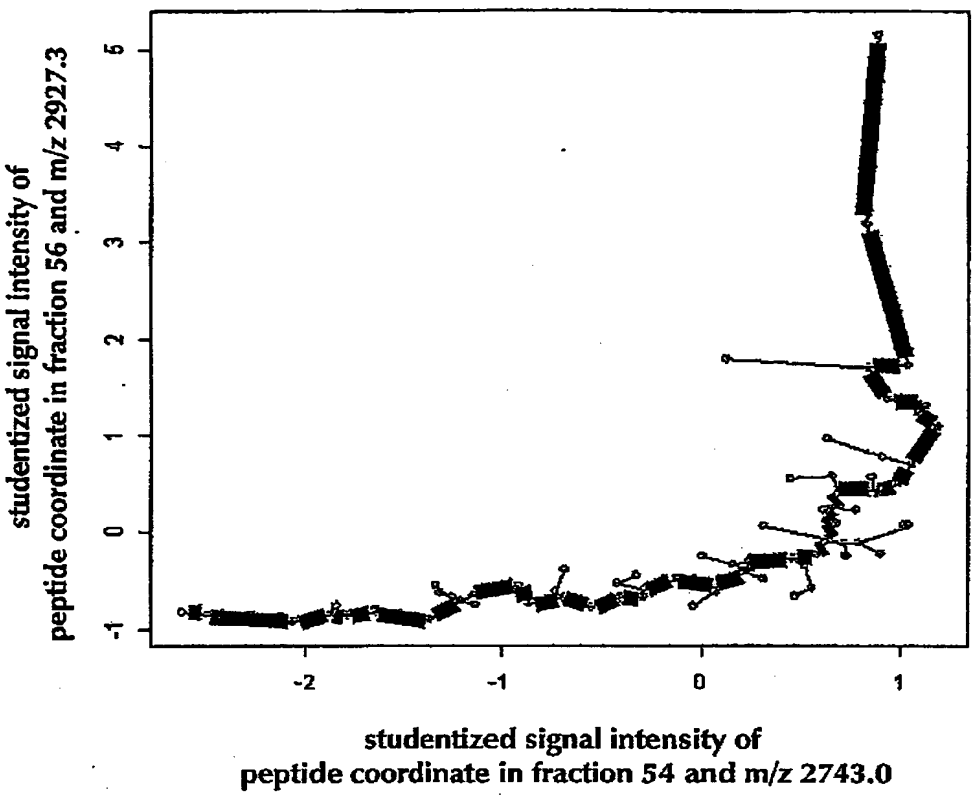
studentized signal intensity of
peptide coordinate in fraction 56 and m/z 2927.3
-1
0
1
2
3
4
5
-2
-1
0
1
studentized signal intensity of
peptide coordinate in fraction 54 and m/z 2743.0

Fig. 25c

	n			
m/z	1	2	3	4
1371.5	-1371.5	0.0	1371.5	2743.0
2927.3	183.3	3109.6	6035.9	8962.2

Fig. 26

```
1          10          20          30          40
|          |           |           |           |
MKWVTFISLL FLFSSAYSRG VFRRDAHKSE VAHRTYDLGE

           50          60          70          80
           |           |           |           |
ENFKALVLIA KREAQYLQQC PFEDHVKLVN EVTEFAKTCV

           90          100         110         120
           |           |           |           |
ADESAENCDK SLHTLFGDKL CTVATLRETY GEMADCCAKQ

           130         140         150         160
           |           |           |           |
EPERNECFLQ HKDDNPNLPR LVRPEVDVMC TAFHDNEETF

           170         180         190         200
           |           |           |           |
LKKYLYEIAR RHPYFYAPEL LFFAKRYKAA FTECCQAADK

           210         220         230       238
           |           |           |         |
AACLLPKLDE LRDEGKASSA KQRLKCASLQ KFGERAFK
```

Fig. 27

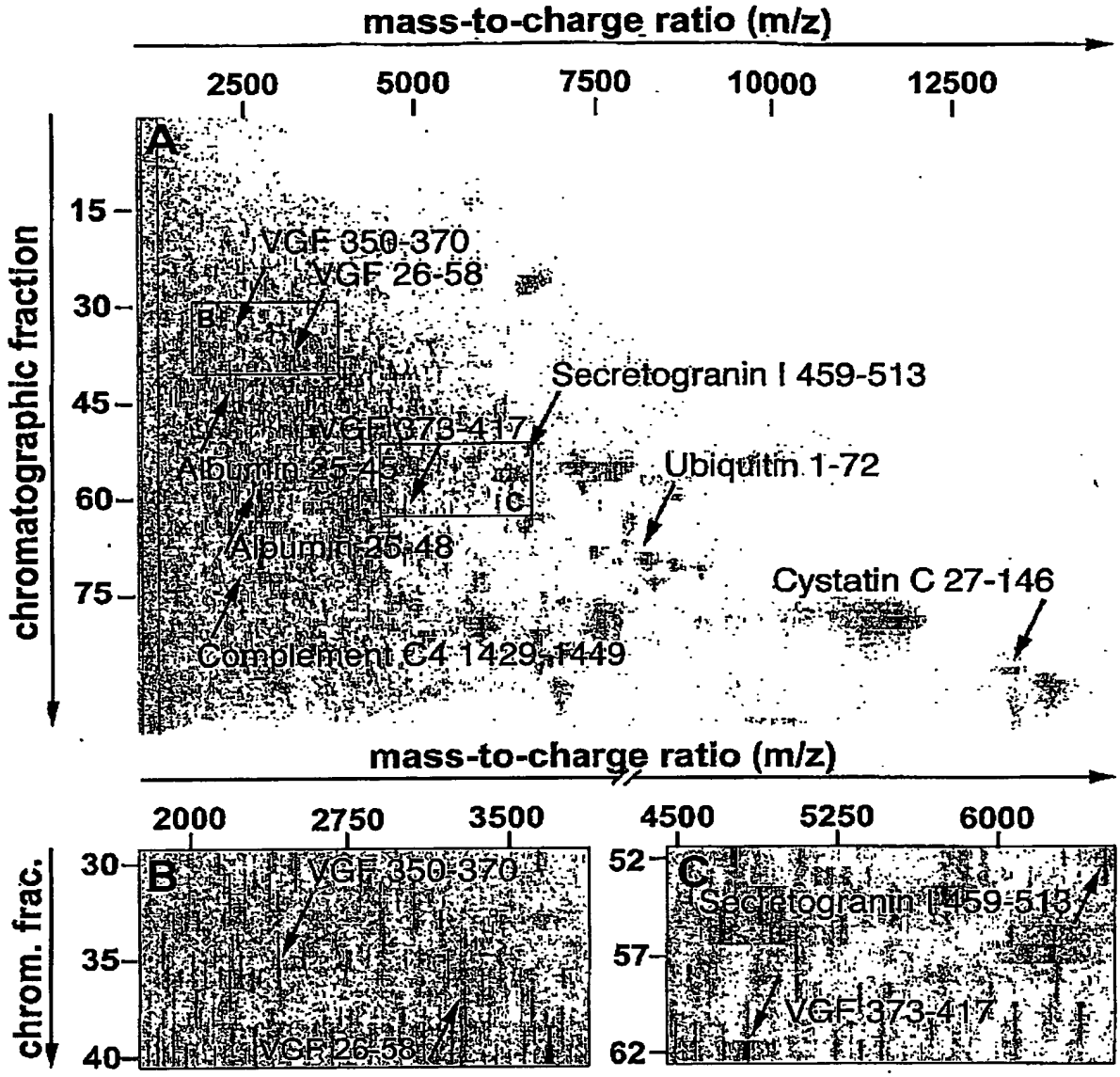
mass-to-charge ratio (m/z)
2500
5000
7500
10000
12500
chromatographic fraction
15
30
45
60
75
A
VGF 350-370
VGF 26-58
Secretogranin I 459-513
VGF 373-417
Albumin 25-45
Ubiquitin 1-72
Albumin 25-48
Cystatin C 27-146
Complement C4 1429-1449
mass-to-charge ratio (m/z)
2000
2750
3500
4500
5250
6000
chrom. frac.
30
35
40
52
57
62
B
VGF 350-370
VGF 26-58
C
Secretogranin I 459-513
VGF 373-417

Fig. 28

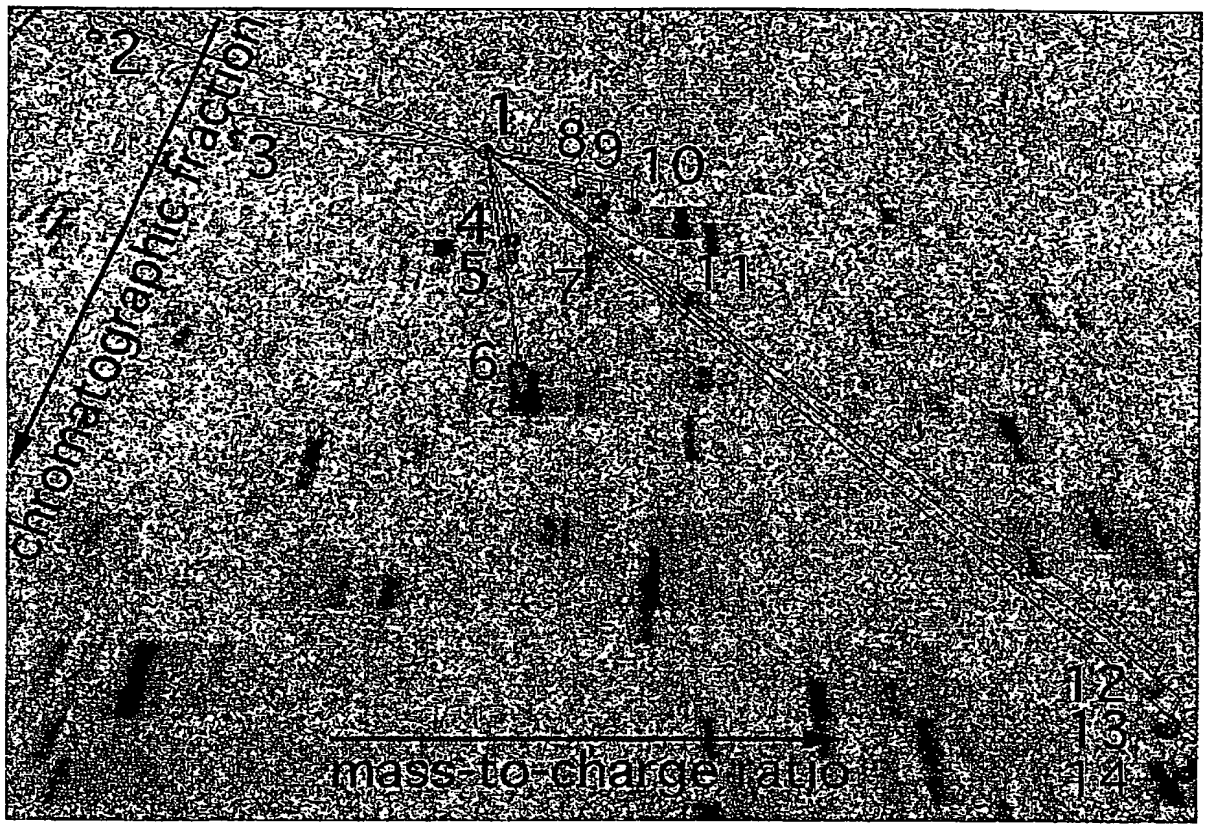
chromatographic fraction
mass-to-charge ratio
1
2
3
4
5
6
7
8
9
10
11
12
13
14

Fig. 29

1 MKALRLSASA LFCLLLINGL GAAPPGRPEA QPPPLSSEHK EPVAGDAVPG PKDGSAPEVR GARNSEPQDE GELFQGVDPR

← # 1 VGF 26-58, Hub Peptide →
← # 4 VGF 26-59, r=0.83 →
← # 5 VGF 26-59, r=0.69 →
← # 7 VGF 23-59, r=0.69 →
← # 8 VGF 26-61, r=0.73 →
← # 9 VGF 26-62, r=0.68 →
← #10 VGF 25-62, r=0.69 →

81 ALAAVLLQAL DRPASPPAPS GSQQGPEEEA AEALLTETVR SQTHSLPAPE SPEPAAPPRP QTPENGPEAS DPSEELEALA
161 SLLQELRDFS PSSAKRQQET AAAETETRTH TLTRVNLESP GPERVWRASW GEFQARVPER APLPPPAPSQ FQARMPDSGP

← → # 2 VGF 177-191, r=0.74

241 LPETHKFGEG VSSPKTHLGE ALAPLSKAYQ GVAAPFPKAR RPESALLGGS EAGERLLQQG LAQVEAGRRQ AEATRQAAAQ
321 EERLADLASD LLLQYLLQGG ARQRGLGGRG LQEAAEERES AREEEEAEQE RRGGEERVGE EDEEAAEAEA EAEEAERARQ

3 VGF 350-370, r=0.76 ← →
← #12 VGF 373-417, r=0.73 —
← #13 VGF 373-417, r=0.68 —
← #14 VGF 373-417, r=0.76 —

401 NALLFAEEED GEAGAEDKRS QEETPGHRRK EAEGTEEGGE EEDDEEMDPQ TIDSLIELST KLHLPADDVV SIIEEVEEKR

481 KRKKNAPPEP VPPPRAAPAP THVRSPQPPP PAPAPARDEL PDWNEVLPPW DREEDEVYPP GPYHPFPNYI RPRTLQPPSA

← #12 VGF 485-522, r=0.81 →

561 LRRRHYHHAL PPSRHYPGRE AQARRAQEEA EAEERRLQEQ EELENYIEHV LLRRP

Fig. 30

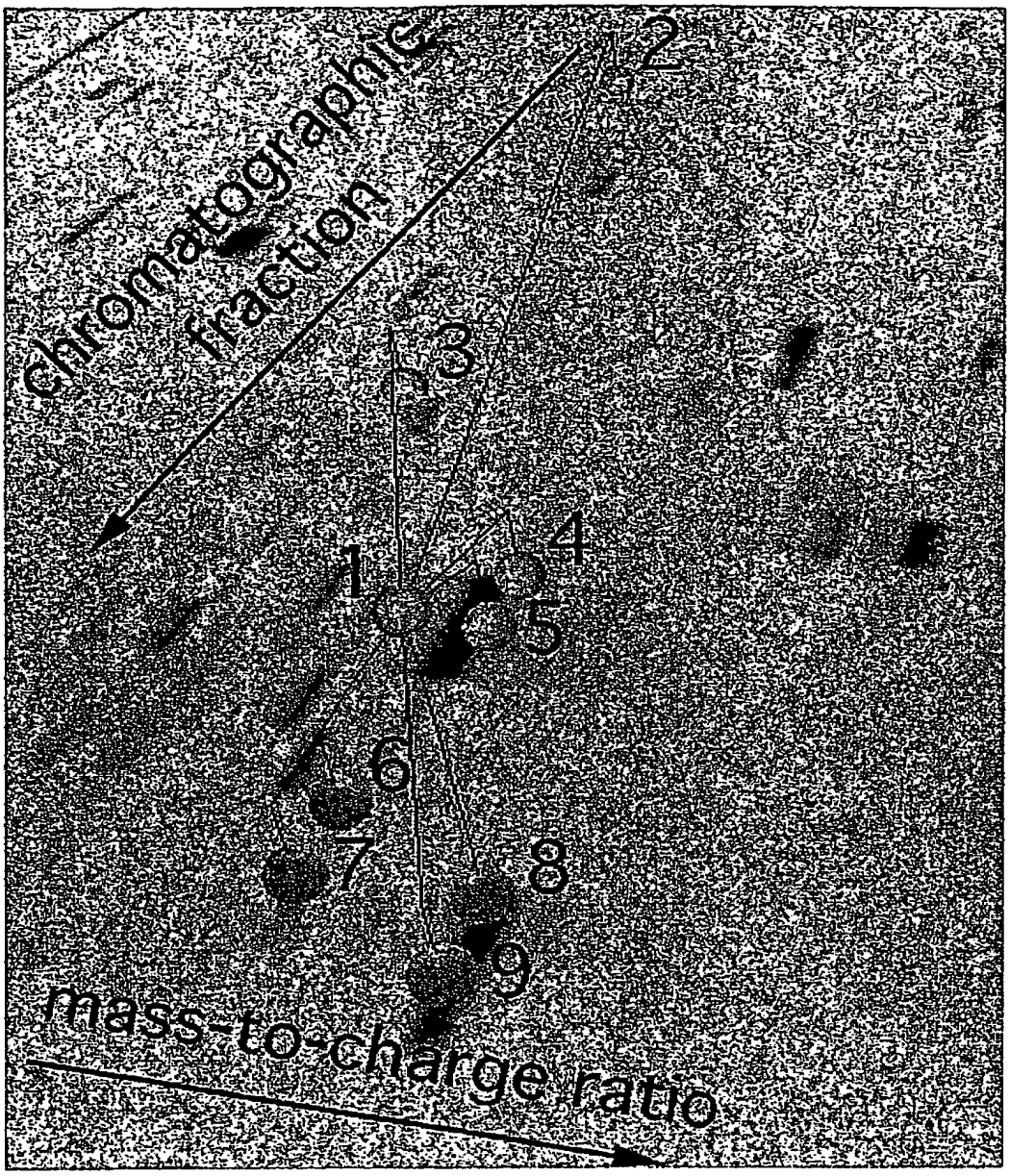

Fig. 31

|r| ≥	Number of ... Correct Precursor Predictions	False Precursor Predictions	All Predictions	Percentage of Correct Predictions
0.95	18	0	18	100%
0.90	40	4	44	91%
0.85	58	10	68	85%
0.80	104	26	130	80%
0.75	178	76	256	70%
0.70	314	192	506	62%
0.65	512	456	968	53%
0.60	756	990	1746	43%
0.55	964	1872	2836	34%
0.50	1186	3086	4272	28%

Fig. 32

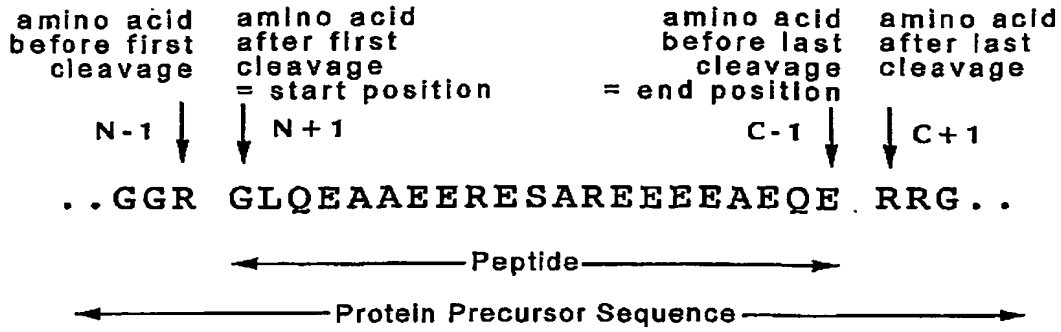
amino acid before first cleavage
amino acid after first cleavage = start position
amino acid before last cleavage = end position
amino acid after last cleavage
N-1
N+1
C-1
C+1
..GGR GLQEAAEERESAREEEEAEQE RRG..
Peptide
Protein Precursor Sequence

Fig. 33

	Number and Percentage of Amino Acid Found at Position of Interest										x-fold Change of Percentages of Amino Acid Compared to General Occurrence			
Amino Acid	n(N-1)		n(N+1)		n(C-1)		n(C+1)		n (Any position)		x(N-1)	x(N+1)	x(C-1)	x(C+1)
A	16	12%	18	13%	16	12%	5	5%	5612	8.2%	1.4	1.6	1.4	0.6
C	1	1%	1	1%	2	1%	0	0%	1109	1.6%	0.4	0.4	0.9	0.0
D	5	4%	22	16%	8	6%	4	4%	3994	5.8%	0.6	2.8	1.0	0.6
E	4	3%	5	4%	30	22%	3	3%	8069	11.8%	0.2	0.3	1.8	0.2
F	5	4%	4	3%	3	2%	7	7%	2195	3.2%	1.1	0.9	0.7	2.0
G	6	4%	12	9%	7	5%	7	7%	4171	6.1%	0.7	1.4	0.8	1.1
H	3	2%	4	3%	2	1%	1	1%	1955	2.9%	0.8	1.0	0.5	0.3
I	0	0%	1	1%	3	2%	2	2%	1592	2.3%	0.0	0.3	0.9	0.8
K	7	5%	8	6%	12	9%	22	21%	4235	6.2%	0.8	0.9	1.4	3.4
L	9	7%	11	8%	10	7%	8	8%	5982	8.7%	0.7	0.9	0.8	0.9
M	5	4%	5	4%	0	0%	0	0%	1120	1.6%	2.2	2.2	0.0	0.0
N	4	3%	4	3%	8	6%	0	0%	2365	3.5%	0.8	0.8	1.7	0.0
P	15	11%	9	6%	1	1%	1	1%	4310	6.3%	1.7	1.0	0.1	0.1
Q	0	0%	7	5%	5	4%	3	3%	3334	4.9%	0.0	1.0	0.7	0.6
R	42	31%	0	0%	9	6%	28	27%	4142	6.0%	5.1	0.0	1.1	4.4
S	4	3%	20	14%	10	7%	3	3%	5154	7.5%	0.4	1.9	1.0	0.4
T	4	3%	3	2%	4	3%	3	3%	3150	4.6%	0.6	0.5	0.6	0.6
V	5	4%	3	2%	6	4%	5	5%	3581	5.2%	0.7	0.4	0.8	0.9
W	1	1%	2	1%	1	1%	3	3%	720	1.1%	0.7	1.4	0.7	2.7
Y	0	0%	0	0%	2	1%	0	0%	1726	2.5%	0.0	0.0	0.6	0.0
Sum	136	100 %	139	100 %	139	100%	105	100%	68516	100%				

Fig. 34

Pair of Amino Acids ...		# of Peptides	% of Peptides	# of Amino Acid pairs in all Precursors	% of Amino Acid pairs in all Precursors	x-fold Increase
... Before Amino-Terminal Cleavage	RR	18	12.9%	398	0.58%	22.2
	KR	13	9.4%	351	0.51%	18.2
... After Amino-Terminal Cleavage	DA	15	10.8%	170	0.25%	43.4
	GR	5	3.6%	215	0.31%	11.4
... Before Carboxy-Terminal Cleavage	QK	7	5.0%	169	0.25%	20.4
	VN	6	4.3%	188	0.27%	15.7
	GA	6	4.3%	293	0.43%	10.1
... After Carboxy-Terminal Cleavage	KR	16	11.5%	351	0.51%	22.4

Fig. 35

Hub peptide VGF 26-58
* predicts unknown peptide with m/z = 3688.03 as **VGF 26-62** + calculated m/z matches found m/z (prerequisite condition) + same start position as hub peptide (+ 69 bonus points) + R as amino acid after end position (+ 4 bonus points)
* predicts unknown peptide with m/z = 2419.41 as **VGF 350-370** + calculated m/z matches found m/z (prerequisite condition) + R as amino acid before start position (+ 5 bonus points) + RR as amino acids before start position (+22 bonus points)

Fig. 36

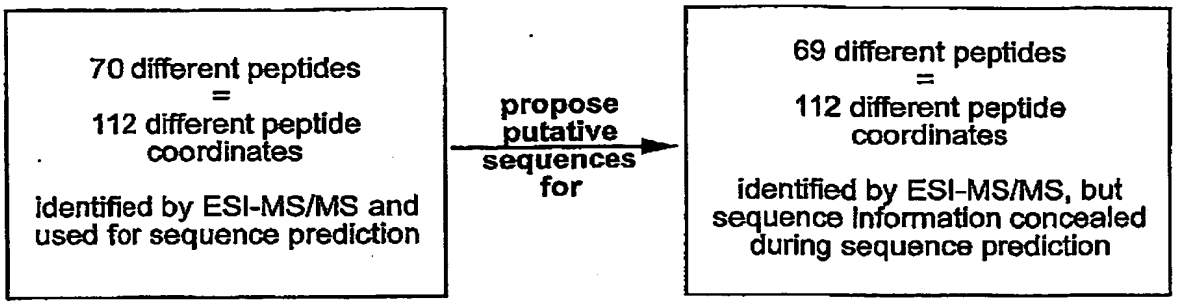
70 different peptides
=
112 different peptide coordinates
identified by ESI-MS/MS and used for sequence prediction
propose putative sequences for
69 different peptides
=
112 different peptide coordinates
identified by ESI-MS/MS, but sequence information concealed during sequence prediction

Fig. 37

$\|r\| \geq 0.75$	Any proposal						Proposal With Most Bonus Points					
	Precursor Correct Start- or End Position Correct		Precursor Correct Start- or End Position False		Precursor False Start- or End Position False		Precursor Correct Start- or End Position Correct		Precursor Correct Start- or End Position False		Precursor False Start- or End Position False	
Model 1: No rules	5	6%	61	75%	15	19%	3	11%	19	70%	5	19%
Model 2: Only Rules Considering Single Amino Acids at Cleavage Sites	13	16%	58	72%	10	12%	10	37%	14	52%	3	11%
Model 3: Only Rules Considering Pairs of Amino Acid Pairs	18	22%	54	67%	11	14%	15	56%	9	33%	3	11%
Model 4: Only Rules Considering Common Start- or End Position	17	21%	51	63%	13	16%	13	48%	11	41%	3	11%
Model 5: Combination: Only Rules Considering Single Amino Acids & Pairs of Amino Acid	19	23%	54	67%	8	10%	18	67%	6	22%	3	11%
Model 6: Combination: all rules	28	35%	45	56%	8	10%	23	85%	1	4%	3	11%

Fig. 38

Correlation to: Albumin	Correlation to: Alb. 25-48	Hub:	Monoisotop. Mass	Sequence
r=0.73		Albumin 25-48	2752.4	DAHKSEVAHRFKDLGEENFKALVL
		related Peptide to Alb. 25-48:		
r=0.80	r=0.77	Albumin 27-50	2750.5	HKSEVAHRFKDLGEENFKALVLIA
r=0.76	r=0.75	Albumin 25-50	2936.6	DAHKSEVAHRFKDLGEENFKALVLIA
r=0.76	r=0.75	Albumin 25-51	3085.5	DAHKSEVAHRFKDLGEENFKALVLIAF
r=0.83	r=0.75	alpha-1-Antitrypsin 397-418	2502.3	LMIEQNTKSPLFMGKVVNPTQK

Fig. 39

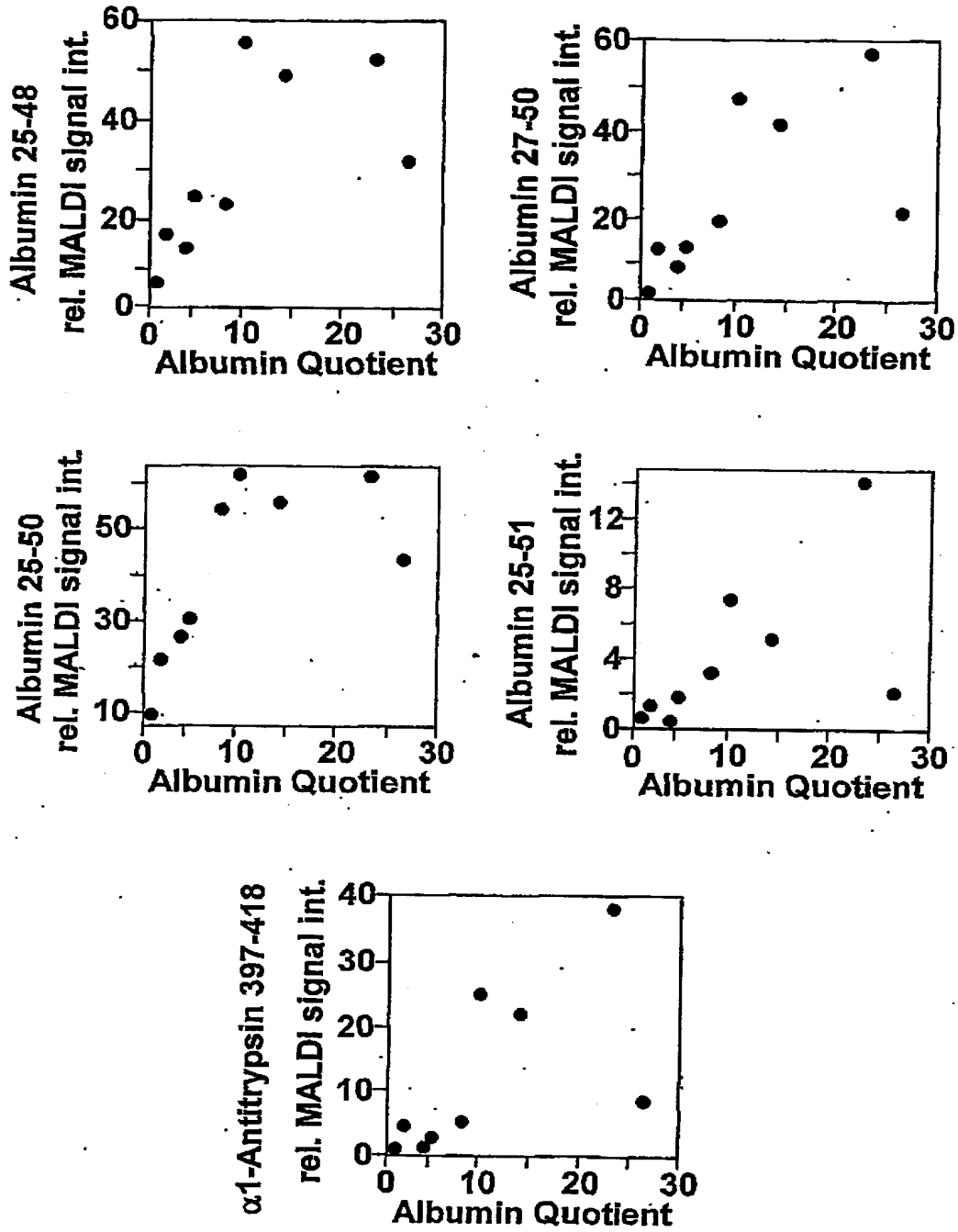
Albumin 25-48
rel. MALDI signal int.
Albumin Quotient
Albumin 27-50
rel. MALDI signal int.
Albumin Quotient
Albumin 25-50
rel. MALDI signal int.
Albumin Quotient
Albumin 25-51
rel. MALDI signal int.
Albumin Quotient
α1-Antitrypsin 397-418
rel. MALDI signal int.
Albumin Quotient

Fig. 40